From THE DEPARTMENT OF MEDICINE, SOLNA
Karolinska Institutet, Stockholm, Sweden

CHRONIC HEPATITIS
MORBIDITY AND MORTALITY IN PATIENTS AND THEIR CHILDREN

Lóa Davíðsdóttir

Karolinska Institutet

Stockholm 2016

Published by Karolinska Institutet.
Printed by Eprint AB 2016

ISBN 978-91-7676-432-9

CHRONIC HEPATITIS
MORBIDITY AND MORTALITY IN PATIENTS AND THEIR CHILDREN

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Lóa Davíðsdóttir

Principal Supervisor:
Professor Rolf Hultcrantz
Karolinska Institutet
Department of Medicine Huddinge

Co-supervisor(s):
Professor Anders Ekbom
Karolinska Institutet
Department of Medicine, Solna

Associate Professor Soo Aleman
Karolinska Institutet
Department of Medicine Huddinge

Associate Professor Ann-Sofi Duberg
University of Örebro
Department of Infectious Diseases

Opponent:
Associate Professor Rune Wejstål
University of Gothenburg
Department of Infectious Medicine

Examination Board:
Professor Per Björkman
Lund University
Department of Clinical Science, Malmö

Associate Professor Karin Pettersson
Karolinska Institutet
Department of Clinical Science Intervention and Technology

Associate Professor Magnus Nilsson
Karolinska Institutet
Department of Clinical Science Intervention and Technology

To my Family

ABSTRACT

Chronic hepatitis, morbidity and mortality of the patients and their children:

The spread of Hepatitis C Virus (HCV) infection started in Sweden in the end of the 1960s with a culmination in the 1970s, most likely due to increased injection drug use. Mandatory notification of acute and chronic HCV infection in Sweden started in 1990. The estimated prevalence of viremic HCV infection in Sweden is 35,000- 45,000. Even though the prevalence is estimated to decrease during the next decade it is believed that the disease burden of HCV-related cirrhosis and HCC will increase. HCV is a global health problem with world prevalence of viremic HCV infection assessed to be 118.9 million. HCV infection has a quiescent progression where most individuals have only mild symptoms until decompensated cirrhosis. Approximately 5-30% patients develop cirrhosis in 20 to 30 years. The natural history of HCV infection in pregnancy and in infants is not well studied.

The aim of this thesis was to study morbidity and mortality of individuals infected with HCV and/or B virus and in children of HCV infected mothers.

In **paper I** the standardized incidence ratios (SIR) for hepatocellular cancer (HCC) were studied in individuals notified with HBV and HBV-HCV dual infection. In the HBV cohort (n=9,646), individuals infected with HBV in 40-49 years had 47 times increased risk of developing HCC compared with general population. In the HBV-HCV cohort (n=1,697), individuals with co-infection for 20-29 years had 34 times increased risk of developing HCC. This established the excessive risk of developing HCC in individuals with HBV and in those co-infected with HBV and HCV compared with the general population. Mortality and cause of death was studied in **paper II**. The standardized mortality ratio (SMR) demonstrated a 6 times excess mortality in the HCV cohort (n=34,235) compared with the general population, and 36 times excess mortality from liver disease. Deaths from illicit drugs and external reasons were common in young adults. In **paper III** the odd ratio (OR) for the outcome of pregnancy of HCV infected mothers, compared with mothers in the general population Mothers with HCV infection (n=9,599) had increased risk for several adverse pregnancy outcomes. They had 7 times excessive risk of their pregnancy terminating in stillbirth and almost two times increased risk for late neonatal death. **Paper IV**, demonstrated excessive mortality in children (n=19,097) of mothers (n=9,599) with HCV infection compared with the general population. From 1 to 4 weeks, the adjusted hazard ratio (HR) was 2.26 and from 1 to 6 months the HR was 2.63. From age 15 to 20 years of age HR was 2.50 and from year 20 and onward the HR was 3.16.

To conclude: HBV and HVB-HCV infected individuals have an excessive risk of developing HCC. Drug related mortality in the HCV-cohort was high. Liver related mortality was high in all cohorts. Mothers with HCV are in risk of adverse outcome of pregnancy. Children of mothers with HCV infection have an increased risk of dying in the perinatal period, within 6 months of living or after their teenage years.

LIST OF SCIENTIFIC PAPERS

The thesis is based on the following original papers, which will be referred to in the following text by their roman numerals (I-IV):

I. **Davídsdóttir L**, Duberg AS, Törner A, Aleman S, Bäck E, Ekdahl K, Blaxhult A, Ekbom A, Hultcrantz R.

   Hepatocellular carcinoma in individuals with HBV infection or HBV–HCV co-infection in a low endemic country. Scand J Gastroenterol. 2010 Aug;45(7-8):944-52

II. Duberg AS, Törner A**, Davidsdóttir L**, Aleman S, Blaxhult A, Svensson A, Hultcrantz R, Bäck E, Ekdahl K.

   Cause of death in individuals with chronic HBV and/or HCV infection, a nationwide community-based register study. J Viral Hepat. 2008 Jul;15(7):538-50

III. **Lóa Davidsdóttir**, Soo Aleman, Anders Ekbom, Matteo Bottai, Ann-Sofi Duberg, Rolf Hultcrantz

   Increased risk for stillbirths in infants born to mothers with hepatitis C, a nationwide study of 19,072 infants
   In manuscript

IV. **Lóa Davidsdóttir**, Soo Aleman, Anders Ekbom, Matteo Bottai, Ann-Sofi Duberg, Rolf Hultcrantz

   Increased mortality in children of mothers with hepatitis C virus infection in Sweden, a national cohort study.
   In manuscript

CONTENTS

LIST OF ABBREVIATIONS

ALT	Alanine Aminotransferase
aOR	adjusted Odds Ratio
CHB	Chronic Hepatitis B
CHC	Chronic Hepatitis C
CI	Confidence Interval
DAA	Direct Acting Antivirals
DR	Cause of Death Register
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
ICD	International Classification of Diseases
IDU	Injection Drug Use
IFN	Interferon
MBI	Body Mass Index
MBR	Swedish Medical Birth Register
NKT	Natural Killer T-cells
NANBH	Non-A non-B hepatitis
ORF	Open Reading Frame
PAR	Swedish Patient Register
PCR	Polymerase Chain Reaction
PHAS	Public Health Agency of Sweden
PI	Protease Inhibitor
PWID	People Who Inject Drugs
RBV	Ribavirin
RIBA	Recombinant Immunoblot Assay
RNA	Ribonucleic Acid

SGA	Small for Gestational Age
SIDS	Sudden Infant Death Syndrome
SIR	Standardized Incidence Ratio
SMI	Swedish Institute for Infectious Disease Control
SMR	Standardized Mortality Ratio
SVR	Sustained Virologic Response
TMA	Transcription Mediated Amplification

1 INTRODUCTION

1.1 HISTORICAL ASPECTS OF HEPATITIS C VIRUS INFECTION

Jaundice has been described since the time of the old testament and then later by Hippocrates in his writings 400 BCE (1). It came apparent when hepatitis B virus (HBV) and hepatitis A virus (HAV) were discovered in the 1970s, that an unknown hepatitis, mostly observed after blood transfusion, existed. In the beginning it was called Transfusion Associated Hepatitis. This new hepatitis was without the usual serological markers of HAV and HBV, and was eventually called non-A non-B hepatitis (NANBH) (2). This sporadically occurring hepatitis proved to have a prolonged, quiescent course, progressing to a chronic hepatitis and liver cirrhosis (3). Finally, identification of the hepatitis C virus (HCV) was successful in 1989 (4, 5). The subsequent development of diagnostic tests showed that more than 90% of NANBH was caused by HCV (4, 6, 7). Most of the cases seemed to develop into chronic infection and gradually, it became apparent that the chronic HCV infection was on a global scale (8).

1.2 HEPATITIS C VIROLOGY

HCV is a small sphere formed virus, approximately 50-80 nm in diameter (9). The virus belongs to the Flaviviridae family and contains a single-stranded positive-sense ribonucleic acid (RNA) genome of 9600 nucleotides (5) (10). There are highly conserved 5' and 3' untranslated regions flanking an approximately 9000 nucleotide single open reading frame (ORF). The ORF encodes a large polyprotein of about 3000 amino acids (11). The protein undergoes posttranslational processing by host and viral proteases to form numerous structural and nonstructural protein and enzymes of the virus. The N terminus encodes the core protein (C), followed by two glycoprotein domains, the envelope E1 and E2, and the pore protein P7 (between the structural proteins and nonstructural proteins). Downstream to this region are the genes encoding nonstructural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B. The nonstructural proteins are involved in the viral replication and packaging of the

viral genome in to capsids formed out of the structural proteins. The organization of the genome is illustrated in figure one (12).

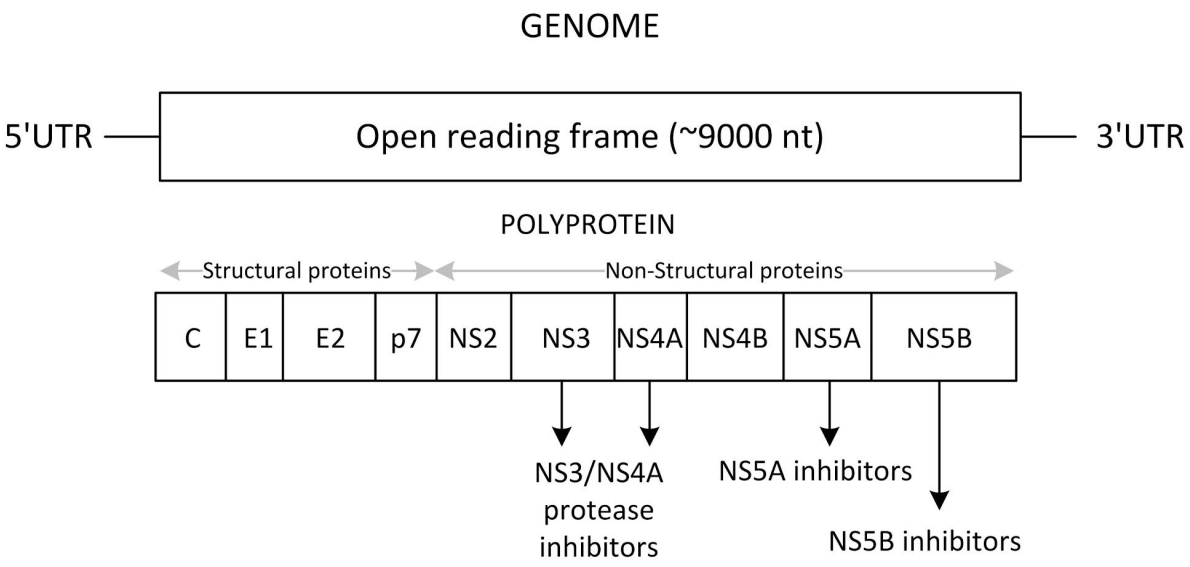

Figure 1. *HCV genome architecture and the targets of Direct Acting Antiviral therapy.*

The virus produces between 10^{11} and 10^{13} virions per day and the halftime is only few hours (13, 14). The HCV strains are now classified into seven genotypes (6 that are clinically relevant) and currently 67 subtypes (15, 16), with individual geographic distribution and sensitivity to interferon-based therapy (17, 18). Despite the difference in the nucleotide sequence among the genotypes, all currently known HCV genotypes are hepatotropic and pathogenic.

1.3 DIAGNOSIS OF HEPATITIS C VIRUS INFECTION

HCV infection is characterized by the appearance of the following markers in chronological sequence HCV RNA, HCV antigens and subsequently HCV antibodies.

1.3.1 Serological Assays

Diagnostic methods for HCV became available in 1990. Antibodies to HCV can be detected using several assays, the standard immunoassays are usually performed in laboratories and rapid immunoassays can be performed on test location.

Standard immunoassays are based on recognition of specific anti-HCV antibodies in serum or plasma. These antibodies are linked to various methods for the signaling of a positive test: enzymatic reaction (EIA, also called enzyme-linked immunosorbent assays or ELISA and chemo luminescence assays). These methods have improved through time. The advances in assay performance, particularly of the EIAs, have been termed as “generations” of the assays. These advances are based on the use of different antigens to detect the HCV antibodies. By every generation, the window from infection until detection became reduced. The more specific second generation was established 1991 and like the third generation, these tests detect human antibodies against HCV antigens from the core, NS3, NS4, and NS5 proteins. The tests become positive about eight weeks after exposure. In most patients, seroconverting occurs between two and six months after exposure to the virus (19-21). The currently used EIAs detect HCV–antibodies with high sensitivity and specificity (22). Antibody tests cannot distinguish between persons with ongoing HCV infection and those with a resolved infection. Anti-HCV cannot be detected in those who are recently infected and have not yet developed anti-HCV antibodies. In immune compromised patients, such as patients with advanced human immunodeficiency virus (HIV) infection or on hemodialysis, anti-HCV may not be detectable despite the presence of HCV infection. In these groups of patients, measurement of HCR RNA is required to confirm the diagnosis (23, 24).

Rapid immunoassay tests have been developed to provide opportunities for HCV testing outside of clinical settings.

To confirm the positive anti-HCV antibodies, the more specific recombinant immunoblot assay (RIBA) has proved to be useful. This test is also based on the detection of HCV antibodies and is not correlated to infectivity. To further narrow the window of infection and to detect and quantify an ongoing infection, quantitative enzyme immunoassays to detect

HCV core antigen were developed (25-27). These HCV-core antigen tests correlate well to quantitative HCV RNA analyses but are less sensitive for low levels of HCV-RNA.

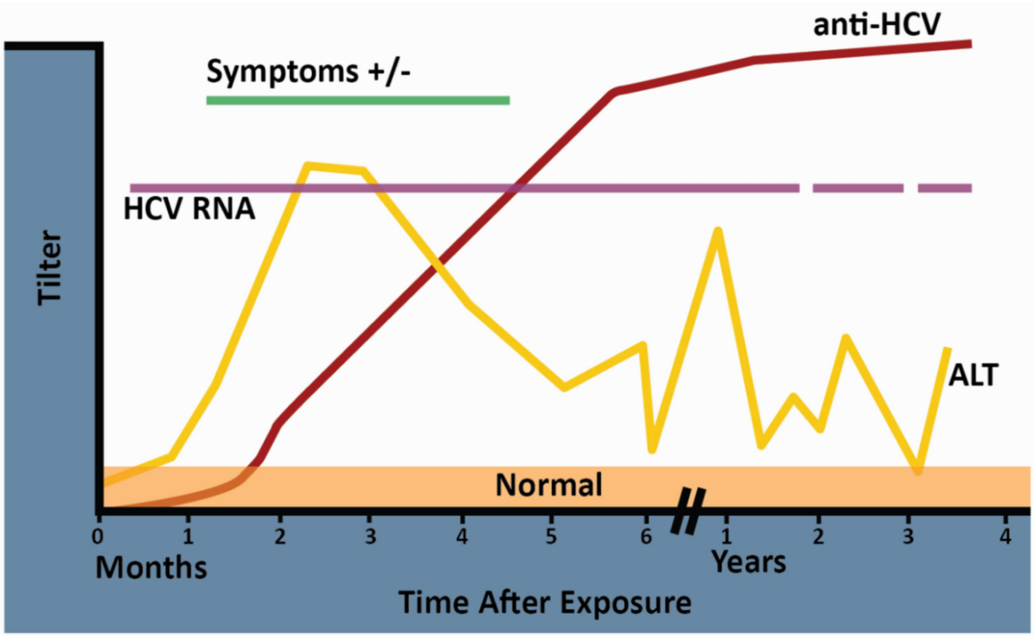

Figure 2. *The serological markers of chronic HCV infection, ALT: Alanine Aminotransferase*

1.3.2 Molecular testing of Hepatitis C

Several methods can be used to detect and measure HCV RNA: Polymerase chain reaction (PCR) based methods, transcription mediated amplification (TMA) and branched DNA assay (28). HCV RNA can be detected in the plasma as early as one week after the time of infection. The levels of HCV RNA can fluctuate around the time of the seroconversion (29). Recently, fully automated real-time PCR or real-time TMA assays (28, 30) have emerged. The newer methods are more sensitive than the previous ones (26). The lower limit of detection of HCV RNA is 10-20 IU/ml and slightly lower for TMA. The range of variation of detection more than ±0.2 Log 10 IU/ ml is considered as a significant difference (26). The unit IU/ml is now used instead of the earlier non-standardized unit copies/ml (31).

Viral sequence analysis has been performed to identify the genome of HCV. Its strains are classified into seven genotypes (1–7, first 6 have clinical importance) and large number of

subtypes (15). Currently, different genotypes require different treatment regimen and affect the duration of treatment. Furthermore, the genotype (and other factors such as previous treatment attempts, resistance to NS5A inhibitors and stage of fibrosis) is of importance when predicting the result and resistance to therapy.

1.4 EPIDEMIOLOGY

1.4.1 The global burden of Hepatitis C virus infection

The major burden of HCV is related to the sequel of chronic infection. HCV infection is an important health problem. The prevalence reports vary depending on regions, time periods and if prevalence of anti-HCV or HCV RNA were used for the assessment.

According to available data, the estimated world prevalence was reported to be from 2.2 to 3.0% (130-184 million people) (32-34). A recent study, including published data from 138 countries between years 2000 and 2015, assessed the global prevalence of anti-HCV positive individuals to be 2.5% (177 millions). The viremic rate was estimated to 67%, corresponding to approximately 118.9 million individuals being HCV RNA positive (33). There is large variability of prevalence in different regions. The highest prevalence is reported from Africa, Asia (0.9-6%) and Eastern Europe (3.1%) and the lowest from industrialized nations in North America, Northern and Western Europe, and Australia (0.5-1.8%) (33, 35, 36). There has been some change over time of the regional estimates, but according to the Global Burden of Disease (GBD) for HCV report of year 2004, the estimates have remained similar since the 1990's (37). The more recent data from Petruzziello et al. observed a decrease of the prevalence in industrialized countries and a considerable increase in low income areas such as Central Africa and Central Asia compared to the period 1990 to 2005 (33).

The most prevalent HCV genotype worldwide is genotype 1 (49.1%), followed by genotype 3 (17.9%), 4 (16.8%) and 2 (11.0%) (33). The remaining 5% were genotypes 5 and 6. In Europe and America genotype 1 is the most prevalent. In Asia genotype 1 was the most

prevalent and in Africa genotype 4 and 1 was the most common genotype. In North and South America and in Australia genotype 1 and 3 dominated. While genotypes 1 and 3 are common worldwide, the largest proportion of genotypes 4 and 5 seem to be in lower-income countries (33).

In people who inject drugs (PWID), genotype 1 is the most prevalent as well. In Europe, genotypes 1, 3 and 4 are highly prevalent among PWID, in Asia genotype 2 and 6, and in Africa genotype 1a and 4 are the most observed. Overall, when comparing with the general population, there is a lower prevalence of genotype 1b in the PWID population and higher prevalence of genotype 1a and 3 (38).

1.4.2 Route of transmission

In the years leading to 1992, the most important source of infection was contaminated blood and blood products and IDU. Transfusion of contaminated (HCV-RNA positive) blood, or blood products, is highly infectious, and 98% of recipients get infected. Even low viral levels (HCV-RNA <100IU/ML) are sufficient (39, 40). Since routine blood donor screening for anti HCV started in the early 1990s in many countries (in Sweden during 1991), the transmission via contaminated blood has decreased drastically and is now virtually eliminated (41). Transmission via unsafe injections in health care is less frequent in industrialized countries, but is still a major problem in other parts of the world (Middle East, South-East Asia and Western Pacific) where the cause is re-using of contaminated needles/syringes and blood products in various procedures (32, 42). One of the largest known outbreaks was the use of contaminated injections in Egypt until the year 1986, in treating schistosomiasis (43). In 1993 and early 1994 patients in Sweden were treated with contaminated immunoglobulin preparation which resulted in increased HCV transmission (44). It has been estimated that 2 million patients get infected annually from contaminated health care injections (45). Organ donation has been another known transmission route (46). Currently IDU is the most

common route of transmission. It is estimated that more than 67% of all PWID get infected with HCV (47, 48). PWID have the highest ratio of new HCV infections worldwide (49).Transmission via sexual activity is a controversial issue but the overall risk seems to be very low (50). The risk of vertical transmission is the most important route of infection among children (51). Vertical transmission is thought to occur in late pregnancy, at delivery or in the postnatal period (51, 52). In the majority of infected infants, HCV RNA levels only became detectable several weeks after birth, suggesting perinatal infection (either late intra uterine or during birth) (53-55). A recent meta-analysis of Benova et al. showed risk of 5.8% in HIV negative RNA positive mothers (56). However, in antibody-positive /RNA-negative mothers, the risk was negligible. The risk in HIV positive and RNA positive mothers was 10.8% (56). HCV viremia in the mother seems to be conditional for vertical transmission. The risk of transmission increases with high viral load. Other factors that have been reported to influence the transmission rate are: female gender of infant, prolonged rupture of membranes, and fetal scalp monitoring (57-59). Caesarian delivery is currently not recommended as a risk-reducing intervention (60, 61). There is no evidence of HCV transmission from mother to infant through breast feeding and therefore it is not contraindicated (60). There are no approved antiviral therapies against HCV for use during pregnancy (62).

1.4.3 Hepatitis C virus infection in Sweden

The spread of non-A, non-B hepatitis started in the end of the 1960s with a culmination in the 1970s, most likely due to increased IDU (63). Mandatory notification of acute and chronic HCV infection in Sweden started in 1990. All clinicians and laboratories that diagnose HCV infection are obliged to notify the infection to the Public Health Agency of Sweden (PHAS). Screening of HCV infection in blood donors started in 1991. In 2007, all individuals who during childhood and before 1992 underwent heart surgery, cancer therapy or were admitted to the neonatology department and subsequently got blood transfusion were recommended to

get tested for HCV infection. In 2010, women who had received postpartum transfusions were also included in the screening recommendations (64). This resulted in identification of about 600 anti-HCV positive individuals (64, 65).

There are currently 9,9 million residents in Sweden. Approximately 2,000 cases of HCV infection are reported to the PHAS each year (65). In a recently published report from the PHAS, a total of 64,200 cases of anti-HCV were reported to the agency from 1990 until the beginning of the year 2016 (65). Of those, 5,000 individuals had wrong identification numbers, 15,800 were deceased, 1,300 had emigrated and 200 individuals were missing. The PHAS made the conservative estimation that 15% are spontaneously cleared from the virus (earlier epidemiologic studies have estimated the viremic rate at 77% thus 23% cleared the virus) and that 5,000 individuals had cleared the virus after therapy (65-67). This results in a total of 37,500 individuals with viremic HCV in Sweden. When assuming that approximately 20% of HCV positive individuals are undiagnosed the prevalence of chronic viremic HCV is estimated to 35,000- 45,000 (0.35%-0.45%) (65).

Nearly 70% of the individuals notified with HCV infection are men and the majority (80%) was born in 1950 or later. Approximately 90% of them originate from the Nordic countries (35, 66). Information regarding suspected route of transmission is required in the clinical notifications. The route of transmission has been found to be IDU in approximately 65% cases, unknown in 26% of cases. Recent study on inpatient care of the Swedish HCV cohort revealed that large proportion of the unknown transmission routes was probably IDU related, since many of these patients had prior psychiatric diagnose related to drug abuse (63). Only 2% of cases were sexual transmission and 6% of cases were transfusion of blood/blood products. Other less frequent infection routes are vertical infection, occupational and nosocomial (35). Since the blood-donor screening started, the transmission via blood-products has decreased dramatically (35). Lidman et al established that 90% of injection drug

users in Sweden were anti-HCV positive by the age of 30 and furthermore within 2 years after first injecting drugs almost 50% had antibodies for HCV (47). In Sweden, 45% of HCV infected individuals had genotype 1, 19% genotype 2, 34% are genotype 3 and 2% genotype 4, (67, 68).

According to the Medical Product Agency, approximately 1100 individuals have been treated for HCV in Sweden in recent years, but this figure has increased since introduction of direct acting antivirals (DAAs) in 2014. Since the millennium, 10,000- 15,000 individuals have been treated in total (63, 65). Previously in the IFN era, cure rate of 40-50% was seen, resulting in estimation of a minimum 5,000 cured persons(63).

In the light of new the treatment era, a study based on a predictive model estimated that doubling the number of patients treated with DAAs is needed to reduce liver related deaths by 70% and viremic cases by 55% in Sweden (67). The HCV-related disease burden in Sweden is expected to increase in the next decade primarily because of an aging cohort and the long lag time, unless increased treatment and cure rate (63, 67, 69).

HCV infection has become one of the leading indications of liver transplantation in Sweden. Among patients that undergo liver transplantation, HCV infection is the underlying cause in approximately 25% of cases (70). The Swedish Organ Transplant Database, Scandia transplant, reported that year 2011, 156 liver transplants were performed in Sweden, of whom 22% were anti-HCV-positive (67, 71). In years 1997-2013 a total of 590 patients underwent liver transplantation on the basis of chronic HCV infection (69).

1.5 CLINICAL MANIFESTATION OF HEPATITIS C VIRUS INFECTION

Following HCV infection, HCV-RNA can be detected in the serum about one week after infection (20, 72-75). Several weeks later, the liver related enzymes rise noticeable and peak more than 10-fold higher than the upper limit of the enzyme (20, 74, 76). The majority of infected persons have no or few symptoms and only about 20% develop symptoms of acute

hepatitis. The time from exposure to jaundice is about 7 weeks (75, 77, 78). HCV-related fulminant hepatitis is rare but the risk might be increased in concomitant chronic HBV infection (79-81). Frequency of development of chronic HCV is 75% to 85%, defined as repeated detection of HCV RNA in the serum of the patient for more than 6 months (82, 83). During chronic HCV infection, viral levels of HCV remain generally constant, although fluctuations can occur (84). Advanced age at infection, male gender, African American race, immunosuppressive state, specific HLA types, reduced innate immune response and to some extent genotypes, are factors that are associated with persistence of HCV virus (82, 85-88). Another host factor that may influence the ability to clear the virus is the polymorphism of a chromosomal locus close to interleukin-28B (IL28B) (89, 90). The allele type C/C was associated with clearance rates of approximately 50-55% compared to 16-20 % of those with the more unfavorable allele type T/T (89). Other host factors such as symptom of acute infection, infection during childhood, female sex, presence of specific HLA types, high titers of HCV neutralizing antibodies and the presence of CD4 T-cell response are associated with higher likelihood of spontaneous clearance (85, 91-96)

Although most patients have mild symptoms or are asymptomatic before culmination in end stage liver disease, some patients complain of generalized, nonspecific symptoms such as fatigue, nausea, anorexia, myalgia, arthralgia weakness and weight loss (97). The symptoms do not correlate to the viral load or level of liver fibrosis. They seem to be correlated to the awareness of the infection and appear to improve after successful treatment (98-100).

Liver fibrosis begins at the portal area and extends as a bridge from one portal area to the next (101). With time, fibrosis can progress further with remodeling and encircles the liver lobule culminating in liver cirrhosis. Studies have shown variable rates of development of fibrosis and cirrhosis. Approximately 5-30% develops cirrhosis in 20 to 30 years. The estimation vary, possibly because of different study populations and due to referral bias in tertiary care

centers (102-104). Lower rates have been reported from community based studies (105). Estimates from retrospective studies have been higher than prospective studies. Studies of patients who presented clinically with chronic hepatitis tend to report a more aggressive course with a higher risk of cirrhosis development (103, 106, 107). Lower rates were reported in those of younger age at infection and in women (108-111). Several additional factors appear to be important determinants of prognosis such as ethnicity, alcohol use, obesity, and HBV or HIV co-infections. Baseline inflammation and fibrosis in liver biopsies or by noninvasive measurements is a good clinical predictor. Patients with mild inflammation on a liver biopsy had low annual risk, patients with moderate chronic hepatitis had approximately 5% and patients with bridging fibrosis had approximately 10% annual risk of developing cirrhosis (102, 112, 113). Other associated factors that had negative impact are D-vitamin deficiency (114). Regular coffee consumption is associated with lower rate of disease progression (115). Liver histology is the best indicator to evaluate the grade of inflammation and the stage of fibrosis (102, 116). Non-invasive methods that evaluate the stiffness of the liver such as elastography have proved to be useful (117, 118).

The most frequent complications associated with liver disease are ascites, variceal bleeding, encephalopathy and jaundice. Almost all patients who develop these complications have cirrhosis. The risk of developing hepatic decompensation is estimated at 3.9% per year in patient with compensated liver cirrhosis (119). Once complications of cirrhosis have occurred, liver transplant has been the only effective therapy to prevent liver-related mortality. With introduction of DAAs, patients with more advanced liver disease can now be treated and some patients have improved and been delisted from liver transplantation awaiting list, after successful therapy with DAAs (120).

1.5.1 Chronic hepatitis and Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the seventh most common cancer in women worldwide. HCC is the second most common cancer related cause of death in men and the sixth leading cause of cancer death in women (121). There is a gradual yearly increase of HCC incidence rates and death rates in many parts of the world such as in central-Europe and North-America (122-124). The incidence is threefold in men compared to women (123). Furthermore, there seems to be a shift of age distribution to increased proportion in younger age groups of 45-60 years (125).

The known HCC risk factors are chronic HBV and/or HCV infection and alcoholic liver disease (126). It occurs less frequently in nonalcoholic fatty liver disease, hemochromatosis, alpha1-antitrypsin deficiency, autoimmune hepatitis, porphyria and Wilson's disease.

The rate of developing HCC is associated with the underlying etiological factors, which have a great regional variability. Almost 75 % of global cases are due to underlying chronic HBV or HCV infection (127, 128). The 5-year incidence is 17-30 % in HCV infection and 10 % in HBV infection (126, 129).

Although most cases of chronic HBV related HCC occur in patients who have already developed cirrhosis (85%), HCC can arise in the absence of advanced fibrosis (130). The risk of HCC in HBV infected individuals is greater in individuals with active replication and high viral load (131, 132). However, available data indicates that both treated patients and those that have serologically resolved their infection are still at risk of developing HCC (133). Thus, indicating probable oncogene activation by the HBV virus itself. Occult HBV infection with or without HCV infection might also be a risk factor in the development of HCC, either through accelerating development of cirrhosis or through hepatocellular transformation by oncogenic action. The significance of occult HBV infection in the pathogenesis of HCC is however still unresolved (134).

Most studies on the risk of HCC in HBV infected individuals are performed in high endemic areas. Only a few adequate studies from Europe have determined the incidence HCC in chronic state of HBV infection. The incidence rate of HCC in Asian countries is higher than in low endemic countries especially in the context of cirrhosis (3.7 per 100 person-years compared to 0.2 per 100 person-years) (121). There are several co-risk factors that contribute to increased risk of developing HCC in HBV infected individuals such as male gender, older age, Asian or African origin, high levels of HBV replication, HBV genotype, duration of infection, co-infection with HCV/HIV/HDV, aflatoxin intake and alcohol consumption (125).

HCC in HCV infection occurs almost exclusively in those with cirrhosis and HCV infection accounts for approximately one-third of HCC cases. According to earlier studies, HCV infection is associated with 15-20 fold increase in risk for HCC (125). A Swedish study demonstrated a 40-fold increase of the risk for HCC after more than 25 years with HCV infection (135). The yearly risk of developing HCC once cirrhosis has developed has varied from 1-8% percent (119, 125, 136). After 40 years of estimated infection, the risk of developing HCC was estimated to be approximately 7% (87, 119, 135, 137). The incidence and mortality rates of HCC are increasing in the developed countries (138). The incidence rates for HCC in Sweden have been reported to be gradually decreasing since the 1980's. However, a recent Swedish study has demonstrated that primary liver cancers are underreported in Sweden, probably because due to fewer histological verified diagnoses in recent years (139). With the aging HCV cohort in Sweden, cirrhosis and HCC have become one of the leading causes of liver transplantation in Sweden (63, 65, 71). Risk factors for HCC in individuals with chronic HCV are male gender, co-infection with HBV or HIV, diabetes, obesity and alcohol consumption.

Only few studies have been undertaken in low endemic areas on HBV and HCV co-infection and the risk of HCC. Earlier studies from high endemic areas have indicated that patients

with dual HBV and HCV infection may have a higher rate of HCC compared to patients with mono infection (140-144).

It is recommended that patients at high risk of developing HCC should be included in local surveillance program (145). According to EASL-EORTC guidelines, high risk is defined as; cirrhotic patients with Child-Pugh stage A and B, and patients with stage C awaiting liver transplantation, non-cirrhotic HBV carriers with active hepatitis and patients who have family history of HCC (with variable evidence which depends on regions) and patients with chronic HCV infection and advanced fibrosis F3 (with variable evidence which depends regions) (145). For diagnosis of HCC, a 4-phase multi detector CT scan or dynamic contrast-enhanced MRI is required for non-invasive criteria to be applied according to EASL. While one imaging technique is required for nodules 1-2 cm in diameter, two different techniques are recommended in suboptimal settings. Nodules above 2 cm require at least one positive technique. Diagnosis of HCC should be based on the identification of the typical signs of HCC (arterial hyper vascularity and venous/late phase washout) (145).

HCC is often diagnosed in advanced stage. The median survival ranges from approximately 6-20 months after diagnosis (146). There are currently several treatment options available for HCC. Surgical resection and liver transplantation are considered potentially curative alternatives. Patients that are not eligible for these modalities are considered for therapy with radiofrequency ablation (RFA) and microwave ablation, percutaneous ethanol and acetic acid ablation, transarterial chemoembolization (TACE), cryoablation, radiation therapy and stereotactic radiotherapy. The systemic therapy presently available is molecular targeted therapy (Sorafenib). The treatment modality is determined by the extent of the tumor and on the severity of the underlying liver disease (145).

1.5.2 Hepatitis C virus infection and extrahepatic manifestations

Extrahepatic manifestations are common in association with chronic HCV infection. It has been reported that almost 40% of the patients have one or more extrahepatic complications (147). They can be grouped into several categories:

Hematological disorders: The association HCV infection and essential mixed cryoglobulinemia is established and a high percentage of these patients have a HCV infection (148). Some association between monoclonal gammopathies and HCV infection has been demonstrated and there seems to be an association between HCV- infection and B-cell non-Hodgkin lymphoma (149). Thrombocytopenia and autoimmune hemolytic anemia have been associated with HCV infection (150).

Dermatological: An association between porphyria cutanea tarda and HCV infection has been demonstrated in several studies (151). Leukocytoclastic vasculitis, lichen planus and necrolytic acral erythema have also been observed in association with HCV infection (147).

Musculoskeletal manifestation: Rheumatoid like arthritis and oligo arthritis has been described in patients with chronic HCV infection (147).

Autoimmune disorders: Autoantibodies are common in patients with chronic HCV infection although they often do not seem to have clinical significance (147). Autoimmune hepatitis indicated by expression of autoantibodies can cause a clinical dilemma when determining the primary cause of hepatitis (152).

Renal disease: Glomerular disease (with or without cryoglobulinemia) has been described in association with chronic HCV infection (153).

Endocrinology: HCV infection has been linked to diabetes mellitus in several studies (154). Thyroid disorders are common in patients with chronic HBV infection and was often a side effect of IFN based therapy (155).

Cardiovascular: HCV infection has been associated with adverse cerebral and cardiovascular events (156).

Nervous System: A range of neurological diseases (stroke, myelitis, encephalomyelitis, and seizures), neuropsychiatric diseases (depression, cognitive dysfunction), peripheral neuropathies and myopathies have been associated with HCV infection (147, 157).

1.5.3 Hepatitis C virus infection and mortality

Survival is decreased in individuals with chronic HCV infection compared to the general population. Earlier studies mostly from high endemic areas have indicated that HCV infected patients are more likely to die at a younger age from drug related causes, liver related causes and from HCC (158-164). Even in individuals who have cleared the virus, there is an overall increased mortality mainly due to complications of drug abuse and liver related causes, indicating that lifestyle factors are important causal factors (163, 165). However, mortality is also increased in HCV infected patients who do not have the concomitant risk factors such as drug use and alcohol overconsumption (103, 166).

1.6 HEPATITIS C VIRUS INFECTION IN PREGNANCY AND BIRTH

1.6.1 Hepatitis C virus infection and pregnancy

The natural course of HCV infection during pregnancy is not well understood. The mother's immune system must simultaneously develop tolerance to paternal alloantigen and sustain an active immunity against HCV to shield both the mother and the fetus from infection (167). The prevalence of anti-HCV in pregnant women varies among populations and regions, but does not seem to differ from the respective general populations (168-171). Prevalence in pregnant intravenous drug users is reported to be high (80-90%) (51).

Acute HCV infection is a rare event during pregnancy (172). Studies have shown a highly significant increase of intrahepatic cholestasis of pregnancy (ICP) in anti-HCV positive women (173-175). A recent Swedish study has confirmed that HCV infection is more

prevalent in women with ICP (176). One explanation might be the inflammatory process triggered by HCV infection; another, might simply be because of misclassification of ICP in the case of HCV infected mothers.

During pregnancy in mothers with chronic HCV infection, a reduction and even normalization in the mean alanine aminotransferase (ALT) has been reported during second and third trimester (177-179). HCV viral load seems to increase during pregnancy, usually reaching a peak during the third trimester (177, 179, 180). Exacerbation of chronic HCV infection and rebound increase in ALT levels and worsening of liver histopathology has been reported in the post-partum period as well as reduction of HCV plasma viral load (181-185).

Several studies have assessed the relationship between HCV infections in the mother and the outcome of the pregnancy. The studies have conflicting results. Two studies demonstrate that the rate of cesarean section was higher than in the control group (171, 186). The high rate was explained by the local protocols for HCV infected mothers. A study from USA demonstrated that infants born to HCV-infected women were more likely to be small for gestational age, have low birth weight and were admitted more often to the intensive neonatology department for assisted ventilation compared to random non-HCV mothers and drug-using non-HCV mothers (187). A recent meta-analysis illustrated that an intrauterine fetal growth disturbance was directly associated with maternal HCV infection when adjusted for various co-factors including maternal age, parity, maternal smoking, alcohol abuse, drugs abuse, coinfection with HBV/HIV and preeclampsia (188). Another meta-analysis demonstrated that there was a significant positive association between a preterm birth and HCV infection (189). Natural Killer T (NKT) cells are important for clearance of HCV in acute infection (190). Hurtado and group established that placental HCV infection increases cytotoxicity of NKT cells (191). This could explain both the mechanism of prevention of vertical infection and the adverse birth outcomes in the HCV infected mothers, as the infection of the virus induces NKT

cytotoxicity which produces cytokines and stimulates the innate immune response in the mother and thus contributes to placental damage, intrauterine growth restriction, perinatal mortality and preterm labor. Mor G et al. have shown that a viral infection of the mother induces production of inflammatory cytokines such as Tumor Necrosis Factor (TNF)α, Interferon (INF)γ, Interleukin (IL)-12 and IL-6 and thus activates the maternal immune system and causes placental damage, fetal mortality and preterm birth (192). If this process does not terminate the birth it might sensitize the mother to other infections and promote inflammation in the fetus (193).

Stillbirth is defined by ICD 10, as early fetal death from 22 weeks to 28 weeks of gestation and as late fetal death after 28 weeks of gestation. The rate of stillbirth in industrialized countries declined from 1940, but epidemiological studies have indicated that in recent years the decline has slowed down (194). There is a great variation within high income countries with low rate in for example Norway (2.2 stillbirths per 1000 births) and high rate in the United Kingdom (3.8 stillbirths per 1000 births) (195). In year 2015 there were 3.7 stillbirths /1000 births (died intrauterine or at birth) according to Statistics Sweden. This variation might indicate that a further reduction is possible, although it might also indicate a difference in reporting and registration of intrauterine deaths between countries (196, 197). There have been speculations about how further improvement in prevention of stillbirth could be possible. Common causes of stillbirth are congenital abnormality of the infant and placenta related causes (lesions, abruptions) (198). Factors associated with infection and inflammation are also common causes for stillbirths (195). Both bacteria and viruses have been implicated (193). Other known risk factors are ethnic origin and socioeconomic status. Studies have shown that populations of different ethnicity within high-income countries have significantly higher ratio of stillbirth (199-204). Less formal education (<10 years) was associated with increased odds of stillbirth (205). Maternal obesity, smoking and advanced age was associated with increased rate of stillbirth (205, 206). Alcohol and illicit drug use during

pregnancy are also documented risk factors (205, 207). Drug abuse is associated with the doubling of the risk of stillbirth (205). Primiparity, advanced maternal age and high BMI at birth are important risk factors for stillbirth (208-210). In 30- 60% of cases the causes remain unknown (211). In a Canadian observational study from 2004, intrauterine death was observed in 3.4 % of 145 mothers with HCV infection (212). The impact of HCV infection on stillbirth is still relatively unknown.

1.6.2 Hepatitis C virus infection and infants

During the first year of life, serologic positivity for anti-HCV in the child may represent passively transferred maternal antibodies. The antibodies seem to clear in 95% of infants by 12 months of age. Therefore most guidelines recommend anti-HCV testing after more than 12 months or the use of HCV RNA analyses. Perinatally acquired HCV infection becomes chronic in approximately 80% of cases (59, 213-215). Spontaneous clearance seems to occur predominately in early stages of infection at a younger age and in children with normal ALT levels (215). Almost all children who remain viremic after two years of age have chronic hepatitis (214, 216, 217). Pediatric HCV infection most often presents with mild liver disease and advanced liver disease is uncommon (218).

1.6.3 Hepatitis C virus infection and therapy

The aim of antiviral therapy is to eradicate HCV and prevent the development of advanced liver cirrhosis and its complications. This is attained by the achievement of sustained virologic response (SVR). SVR is defined by the absence of HCV RNA at 12-24 weeks after the end of treatment (219). The positive predictive value of SVR at week 12 is 99% chance of cure (219, 220). Eradication of the virus halts the progress of fibrosis in the liver and improves the liver histology and the general outcome of the patient (104, 221-224).

Historically recombinant IFN- alfa was introduced in 1986 to treat patients with NANB hepatitis (225). Partial effectiveness was noticed and only few achieved SVR. In the late

1990's, Ribavirin (RBV) was added to the regime and SVR increased to approximately 34% after 24 weeks of treatment, and 42% when prolonging therapy to 48 weeks (226, 227). The next step was prolonging the half-life of IFN by pegylation (adding a polyethylenglykol molecule), which improved the virologic response rates and reduced the injection frequency. Weekly injections of long-acting pegylated (PEG) IFN alone for 48 weeks induced SVR in approximately 40%, this increased to approximately 55% when PEG-INF was combined with RBV for 48 weeks (228, 229). The SVR rate was higher for HCV genotypes 2 and 3, and lower for genotype 1. Treatment in patients with cirrhosis was less effective (230, 231). Because of unsatisfactory therapy results in HCV genotype 1 as well as severe side effects, new treatment options were needed (232).

In Sweden at that time, patients with genotype 2 and 3 (and no contraindications) were generally offered PEG-INF based therapy, but for patients with genotype 1, therapy was recommended only for those with progression of liver fibrosis.

Over the past decades, increasing knowledge of the HCV lifecycle has revealed several steps of the viral cycle that could potentially be new targets for therapy. Understanding of the structures of the HCV genome and proteins enabled the design of drugs that led to disruption of the viral replication and subsequently a targeted anti-viral therapy for HCV. The two first generation protease inhibitors (PI) were marketed in 2011. However, when used as monotherapy, resistance mutations developed rapidly, and combination therapy was needed. (233). First generation PI therapy plus PEG-INF and RBV increased SVR by 30% in patients with HCV genotype 1. This INF-based regimen was associated with an increase of adverse events, such as severe anemia, dysgeusia and rash and was contraindicated in patients with severe liver cirrhosis.

In the following IFN-free era with direct-acting antivirals (DAAs), there are four categories of drugs according to their therapeutic target: the nonstructural proteins 3/4A (NS3/4A)

protease inhibitors (PIs), NS5B nucleoside polymerase inhibitors (NPIs), NS5B non-nucleoside polymerase inhibitors (NNPIs) and NS5A inhibitors (234). See figure 1 for schematic overview of the targets of the DAAs.

Currently (2016) in Sweden there are restrictions of whom to treat, according to national treatment guideline (219, 234, 235):

- Patients with liver fibrosis stadium 2-4 (F2-F4) assessed by histology or with elastography.
- Patients that have undergone organ transplantations, patients with extrahepatic manifestations.
- Persons planned for IVF

Choice of DAA treatment regimen is based primarily on the HCV genotype and stage of liver fibrosis and the pricing in Sweden. Also, to consider is the risk of evolving viral resistance against NS3/4A PIs and /or NS5A inhibitors which may be problematic in the few patients who relapse and need re-treatment.

Tests for assessing resistance to DAAs are now available and could be used, according to EASL guidelines, to guide decision regarding therapy (236).

Therapy with DAAs results in 90-100% SVR after 12 weeks (237-247). The lower SVR rates are in patients with advanced cirrhosis and HCV genotype 3, while patients with genotype 1, especially genotype 1b, usually achieve SVR in close to 100%. Today, IFN-free regimens are primarily recommended in Sweden and in the majority of the Western World (219, 236).

1.7 HEPATITIS B VIRUS INFECTION AND HBV-HCV DUAL INFECTION

1.7.1 Hepatitis B

Hepatitis B virus is a DNA virus and belongs to the Hepadnavirus family. When the virus has penetrated into the nucleus of the hepatocyte, the viral DNA exists as a partially double

stranded, circular form. The host cell enzymes then aid in several steps resulting in the completion of the double stranded DNA and covalently closing of the circle to form a highly stable covalently closed circular DNA called cccDNA. During the course of the viral life cycle more copies of cccDNA form in the nucleus becoming a stable reservoir of viral genetic material within the cell. The cccDNA functions as a mini chromosome, a template for the cellular enzyme to produce new pre-genomic messenger RNA (248, 249). The HBV cccDNA molecule binds tightly to the histones and other proteins in the nucleus of the hepatocyte. This, together with the long half-life of the hepatocyte, contributes to a persistent infection (250, 251). The fact that HBV-DNA can integrate into the host DNA might be a co-factor in the development of HCC in non-cirrhotic liver (252, 253).

HBV infection is a major public health issue worldwide, with 2 billion people infected, and 350 million suffering from chronic HBV infection (254, 255). A recent analysis revealed that 3.6% of the general population globally is chronically infected with the virus (WHO) (255). Notification of HBV infected patients has been mandatory in Sweden since 1969. Prevalence in the WHO European region was estimated to be 2.1%, ranging from estimated 0.01% in the United Kingdom to 10.3% in Kyrgyzstan. Prevalence levels of 10% and above persist in some African countries.

In industrialized countries, rates of new infections and acute disease are highest among young adults and transmission predominantly occurs via IDU and high-risk sexual behaviors (256). In Asia and Africa, chronic HBV infection is common and usually acquired perinatally or in early childhood (257, 258). Sweden is a low prevalent country, the overall HBV prevalence has been reported < 5 % and the prevalence of chronic HBV <1% (259-262). Prevalence among in individuals originating in high endemic countries was higher, 6.5% (260). The trend in Sweden for the last decade has been an increases in chronic HBV and a decrease in acute HBV infection(260).

The risk of developing chronic HBV infection decreases with age at infection, from about 90% when infected perinatally up to 6 months of age, to 20–60% between the ages of 6 months and 5 years and to <5% in otherwise healthy adults (255).

The natural course of chronic HBV infection is characterized by the interaction between the replication of the virus and the immune response of the host and can be separated into phases (263-265).

Initially, chronic HBV infection consists of early replicative phase of chronic hepatitis B (CHB), characterized by the presence of hepatitis B e antigen (HBeAg) and high serum levels of HBV DNA (referred to as HBeAg-positive CHB) with active liver disease and elevated ALT levels. However, the majority of patients will clear HBeAg (and produce anti-HBe antibodies) and achieve a state of non-replicative infection, characterized by low or undetectable serum levels of HBV DNA and normal ALT levels.

Perinatally acquired CHB is characterized by a replicative phase and immune tolerance. Initially, the HBV replication increases, the hallmark being the presence of HBeAg and high levels of HBV DNA in the serum. No clinical symptoms are apparent, the level of ALT is normal, and there are no signs of active liver disease (266, 267). This phase can last 10-30 years. During this time there is low rate of spontaneous HBeAg clearance.

During second or third decades after infection, transition from the immune tolerance phase to immune clearance usually takes place (268). During this phase spontaneous HBeAg clearance takes place. This phase is often accompanied with decrease in HBV-DNA and an increase in serum ALT, which is believed to be caused by lysis of hepatocytes due to immune reaction (269). This immune activation phase is usually followed by a low replication phase or inactive carrier state with negative HBeAg and positive anti-HBe. The quantification of HBV-DNA and the ALT concentration are low. Significant liver fibrosis is rare in this phase

(270). To be categorized as inactive carriers the blood levels of ALT and HBV-DNA need to be stable for at least a one year period.

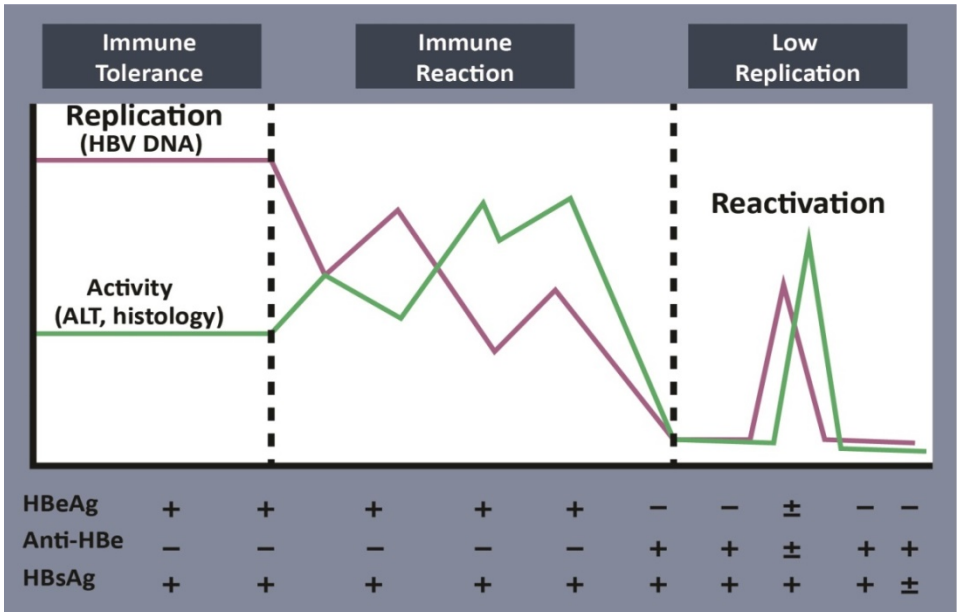

Figure 3. *Course of chronic HBV infection*

Patients with a prolonged immune activation phase are at higher risk of liver damage and should be considered for treatment of the HBV infection. Decision of antiviral therapy is based on assessment of the risk for progressive liver disease, using stage of fibrosis (liver biopsy or elastography), blood ALT level and the HBV DNA level to support the decisions (271). The antiviral therapies currently recommended for the treatment of chronic HBV infection are PEG-IFN for 48 weeks alternatively therapy with one of the nucleoside or nucleos(t)ide analogues (NAs), telbivudine, entecavir, or tenofovir for indefinite time (271). The available NAs are potent in achieving sustained viral remission, but a total cure is rare, due to persistence of transcriptionally active viral cccDNA in the nucleus (271).

Since 1982, safe and effective vaccines against HBV infection have been available

1.7.2 HBV-HCV dual infection

HCV and HBV share a common route of transmission and both are able to induce a chronic infection that can lead to fibrosis and cirrhosis and HCC (127). In highly endemic areas, co-

infection is not uncommon among subjects with high risk of parenteral infection (272). The risk of persistence of hepatitis B genome in individuals negative for HBV surface antigen (occult HBV) is higher among individuals with HCV infection (273-275). The estimated prevalence of HBV-HCV dual infection is 5 to 20 % in HBsAg positive patients and 2-10% in anti-HCV positive patients with variable geographic distribution (276). The prevalence of dual infection in Sweden is low.

2 AIMS

The overall aim of this thesis was to study morbidity and mortality of individuals with chronic hepatitis and of their children. The specific aims were to:

- To study the association between chronic HBV infection and combined HBV-HCV infections and HCC (paper I).
- To study mortality and cause of death in all individuals with diagnosed HCV infection, chronic HBV infection or dual HCV-HBV infections in Sweden (paper II)
- To study the association between HCV infection in mothers and outcome of their pregnancy and birth (paper III).
- To estimate the mortality of children to mothers with HCV infection. Furthermore, to study the cause of death of these children (paper IV).

3 MATERIAL AND METHODS

3.1 THE REGSTRY SETTINGS

The Swedish personal identification number (PIN) was introduced in 1947, covering the total resident population of Sweden. The PIN is unique for all residents and is issued by the Swedish Tax Agency at birth when the child is registered in the Census register. A PIN is also issued to all immigrants registered in Sweden. The PIN is used in all health care, social services, and other authority contacts.

Statistics Sweden was founded in the middle of the 18th century, derived from old Swedish church registers. It has since then been responsible for public statistics regarding decision making, debate and research in Sweden. From Statistics Sweden we obtained information on duration of formal education and of economical support of the study objects in study III and IV.

A notification of all newly diagnosed HBV and HCV infections to the Public Health Agency of Sweden (PHAS) has been mandatory, since 1969 and 1990, respectively. The notifications are registered at the PHAS using the personal identification number of the patient. Two parallel notifications are made to the PHAS, one from the clinician and the other from the laboratory that diagnosed the infection. From the laboratories all results indicating an infection are reported, positive HBsAg and/or HBV–DNA, positive HCV antibodies and/or HCV–RNA analyses. The clinical notification contains information of epidemiological interest if known, such as route of transmission and information regarding whether the HBV infection is considered acute or chronic. In the case of an HCV infection, the registration does not differentiate acute from chronic.

3.1.1 The National Tax Board

The National Tax Board provides dates for emigration and immigration, deaths, and country of birth of all Swedish residents.

3.1.2 The Swedish Health registers

The National Board of Health and Welfare (NBHW) is a government agency that holds several registers such as the cancer register, the cause of death register, the patient register and the medical birth register, among others.

3.1.2.1 The Cancer register

Notification of all cancers in Sweden to the Cancer Register has been mandatory for both clinicians and pathologists/cytologists since 1958. Required information in the report is the site of the tumour, histological type, date of diagnosis, identification number for the tissue specimen, and the PIN. Since 2005 the cancers are coded according to the third version of International Classification of Diseases for Oncology, ICD-O/3, but all cancer diagnoses are also, since 1958, coded according to the seventh revision of the International Classification of Diseases (ICD-7). The Swedish Cancer Register is complete and comparable to other quality registers in Europe (277). Earlier studies have verified that over 95% of all tumours are reported. Furthermore, around 99% have been histologically or cytologically verified (278). However, a recent Swedish study demonstrated that 37%-45% of HCC in Sweden were never reported to the Cancer register. The reason was probably that fewer cases of HCC were histologically verified because of improved non-invasive diagnostic methods, and the absence of a histology report resulted in missed cancer reports (139).

3.1.2.2 The Cause of Death Register

The cause of death register (DR) contains information on all deaths of Swedish residents since 1961. For each death the PIN, sex, date of birth, date of death and the main and up to 19 underlying causes of death is recorded. The causes of death were coded according to ICD-8 in 1969-1986, ICD-9 in 1987-1996, and ICD-10 was implemented in 1997. The DR is updated every year. Until 2011, the register covered all deceased persons who at the time of death were registered in Sweden, regardless of whether the death occurred within or outside the country. From year 2012 and onwards all deaths in Sweden were included whether the

deceased were Swedish residents or not. The Cause of death is collected from the death certificate and is defined as the disease or injury that initiated the chain of disease that finally resulted in death. The register has 98-99% completeness (279). Different factors affect the completeness of reporting. It seems to be more complete in younger patients and when the cause of death is violent(280).

3.1.2.3 The Swedish National Patient Register

The Swedish National Patient Register (PAR) contains information on inpatient care since 1964 (nation-wide since 1987). Psychiatric care was included from 1973. Each in-hospital episode is recorded with dates of admission and discharge, surgical procedures, and the discharge diagnoses with up to eight medical conditions coded according to ICD. The eight revision (ICD-8) was used years 1969-1986, the ninth revision (ICD-9) was used in years 1987-1996 and the tenth revision (ICD-10) from year 1997. From year 2001, information on outpatient visits including day surgery and psychiatric care from specialized outpatient care units, are registered in the Outpatient Register. More than 99 % of all somatic and psychiatric hospital discharges are included in the in the Inpatient Register. Ludvigsson et al., 2011, revealed a high validity for many but not all of the diagnoses. Positive predictive value was 85%-95% of diagnoses in the register (281).

3.1.2.4 The Medical Birth Register

The Swedish Medical Birth Register (MBR) was founded in 1973. It is mandatory for all health care providers to report to the register. The number of infants born each year varies between 86.000 and 120.000. Information about all births is collected from medical records from prenatal, delivery, and neonatal care providers. It includes information on the mothers' previous diagnoses, diagnoses during pregnancies, length of pregnancy, mode of delivery, diagnoses of the children and information about various parameters of the children at birth. The quality of the register is good. Of all newly born children, 97-99% percent is reported to

the register(282). The loss of information over birth is approximately 0.5-3 percent per year (283).

3.2 STUDY POPULATION AND METHODS

All four studies were register based. The study populations were identified from the national surveillance database of HCV and HBV infections at the PHAS. The National Tax Board (paper I and II) reviewed the cohorts and excluded all duplicates and notifications with incorrect PINs. The Swedish HCV cohort has been well characterized in earlier studies, adding data from the Swedish national registers and thus making the cohort very suitable for epidemic analysis (135) (284, 285)

3.2.1 Paper I

All HBV- and HCV- notifications in the years 1990 to the end of 2004 were identified. In the HBV cohort all the notifications for acute hepatitis B and those with HCV co-infection were excluded. In addition, all individuals who were reported to have been infected in adulthood were excluded. All HBV notifications were matched with the HCV notifications from 1990 to 2004 to identify co-infected individuals. After exclusion of all notifications of acute HBV infection, the remaining ones were included in the HBV–HCV co-infection cohort. The study population consisted of two cohorts; the HBV-cohort consisted of 9,646 subjects with chronic HBV infection and the HCV-HBV co-infection cohort consisted of 1,697 patients.

3.2.2 Paper II

All HBV- and HCV-notifications from years 1990 to 2003 were identified. All notifications of acute hepatitis B were excluded. The study population constituted of three cohorts; the HCV-cohort consisted of 34,235 individuals reported for HCV-infection (no HBV infection), the HBV-cohort of 9,517 individuals reported with chronic HBV infection (no HCV infection), and the HCV-HBV co-infection cohort consisted of 1,601 individuals with both HCV and chronic HBV-infection.

3.2.3 Paper III

All HCV notifications to the PHAS years 1990-2011 were identified. Identification of all childbearing women with HCV infection and their infants was obtained by linking information on all women notified with HCV infection to MBR by using their PIN. The medical birth register then matched the infants to five controls, i.e. infants of mothers without HCV infection. The matching criteria were birth year, gender, county of origin and age of the mother. Because of the number of matching criteria, the total (mean) numbers of controls per case were only 4.5. The study population constituted of 19,072 infants of 9,599 mothers reported with HCV during 1990-2011. They were compared with 86,164 infants of 83,986 mothers without HCV infection.

3.2.4 Paper IV

As in paper III, the study population consisted of all children of women notified with HCV infection in the National Surveillance Database at the PHAS years 1990 to 2011. These children were matched with five controls, i.e. infants of mothers without HCV infection. The matching criteria were birth year, gender, and county of origin and age of the mother. Because of the number of matching criteria, the total (mean) numbers of controls per child were only 4.5. The study population constituted of 19,097 offspring of 9,599 mothers with HCV infection compared with 86,192 children of 83,986 mothers without HCV infection.

3.3 ANALYSIS

3.3.1 Paper I

The study period was 1990-2004. The PINs of individuals in the HBV and HBV-HCV co-infection cohorts were used to link to the Cancer register. The National Tax Board added information on dates of emigration, immigration, deaths, and country of birth.

To avoid overestimation of the risk due to surveillance bias, the time of observation commenced three months after the date of HBV notification for each subject in the HBV

study population. For the HCV–HBV co-infection cohort, the observation time commenced three months after the date of the second of the two notifications. The observation time terminated either at death, the first date of HCC diagnosis reported to the Cancer Registry, or 31 December 2004, whichever came first.

Chronic HBV infection in Sweden is mostly found in immigrants from high endemic areas (259, 260, 262). 90% of all patients that are infected with HBV during the perinatal period develop chronic infection. We therefore stratified the cohort according to age; less than 30 years, 30 to 39 years, 40 to 49 years , 50 to 59 years, and 60 years and older. Earlier epidemiological studies have demonstrated that the HCV-HBV co-infection cohort is similar to the HCV cohort in Sweden concerning transmission route. We therefore estimated time of infection using a model developed in earlier comparable studies on the HCV cohort (135, 284). Time from infection was assessed for all routes of HCV infection based on available epidemiological data in Sweden (286-288). For transmission route notified as IDU, unknown or sexual transmission the year of infection of persons born before 1930 were considered to be in 1965; the age of infection in persons born in 1930 were considered to be at the age 35 years, then falling linearly so when born in 1955 or later they were considered infected at 20 years of age. We used the age at the time of notification for patients younger than 20 years. We approximated the year of infection to be 1980 for persons with transfusion-associated HCV infection before blood donor screening was introduced in 1991. Date of notification was used as the date of infection for persons with nosocomial and occupational route of transmission. We then made three strata depending on the time with infection: infection for less than 20 years, infection between 20 and 30 years, and infection for more than 30 years. We then assessed the risk of hepatocellular cancer (HCC) in the study cohorts by comparing with the general population. The expected number of HCCs was calculated on the basis of age, sex, and calendar year-specific cancer incidence rates from the Cancer Register, and the observed number of person years in the HBV and the co-infection cohorts. We calculated

standardized incidence ratios (SIR) of HCC by comparing the expected incidence with the observed HCC incidence. Ninety-five percent exact confidence intervals (CIs) were calculated assuming a Poisson distribution of the number of observed cancers.

3.3.2 Paper II

The study period was 1990-2003. The PINs of individuals in the HCV and HBV cohorts were used to link to the DR. The National Tax Board added information on dates of emigration, immigration, deaths, and country of birth.

For each subject, the observation time started six months after the HBV or HCV notification (first notification if co-infected) and ended at death, emigration, or the end of study, whichever occurred first. To avoid overestimation of the risk due to surveillance bias from HBV and/or HCV infections diagnosed as a result of the disease that led to death, all individuals who died less than 6 months after the hepatitis notification were excluded.

ICD codes according to the main ICD-chapters were used to categorize cause of death in main groups. ICD codes of special interest (liver related, drug and alcohol related, external reason, HIV, NHL, MM and other malignancies) were then further analyzed. The standardized mortality ratio (SMR) was calculated by comparing the mortality in the study population with the mortality in the general population. The observed number of deaths was divided by the expected number of deaths. The mortality rates for the general population were obtained from the DR. For the calculation of the expected number of deaths, the sex and age-specific mortality rates in the calendar year 1999 were used.

3.3.3 Paper III

The study period was 1973-2011. We used relevant ICD codes from ICD-10 (1997-2011), ICD-9 (1987-1996) and ICD-8 (1973-1986) for the studied outcomes. Information about pre-pregnancy diagnoses of the mothers, such as diabetes, hypertension, alcohol and drug use, was obtained from both the PAR and the MBR. From Statistics Sweden, we obtained

information on the number of years of formal education completed at the time of birth of the child, categorized as: less than 9 years, 9 to 12 years, more than 12 years. Country of birth was categorized into: Sweden, other Nordic country, non-Nordic European country and non-European country. Cigarette smoking at any visit to the maternity care was reported as daily smoking. Women were categorized by whether they were living with the child's father, not living with the father, or other family situations. Women were categorized as lean if body mass index (BMI) at first antenatal visit was 11.0- 19.9 kg/m^2, normal weight if BMI was 20.0- 24.9 kg/m^2 , overweight if BMI was 25-29.9 kg/m^2 and obese if BMI was 30.0-60.0kg/m^2. Parity was divided into 1, 2 or 3+ children. Data on complications of the pregnancy (preeclampsia, gestational diabetes, gestational hypertension), were obtained from the MBR.

Information on outcomes regarding the infant was obtained from the MBR such as Apgar score, birth weight and gestational age. Apgar score at 5 minutes after birth was used, categorized into 0-6 and 7-10 (used in earlier comparable studies (289)). Gestational age at birth was categorized into: very preterm (<32 weeks), moderately preterm (32-36 weeks), and term (≥37 weeks). The infants were then categorized if they were small for gestational age (SGA). SGA was defined as birth-weight less than two standard deviations (SD) below the mean for gestational age based on Swedish reference curve of estimated fetal growth (290). Low birth weight was defined as <2500 grams. Gestational age at birth was categorized into: very preterm (<32 weeks), moderately preterm (32-36 weeks), and term (≥37 weeks). For outcome of interest, relevant ICD codes from ICD-10 (1997-2011), ICD-9 (1987-1996) and ICD-8 (1973-1986) was used. Information on malformation was obtained from the MBR and from the PAR. Further information on complications at birth, such as cephalohematoma, neonatal seizure and intraventricular haemorrhage was obtained from the MBR. Information over intrauterine death and postpartum death was obtained from the MBR, which retrieved the information from the DR. Stillbirth was defined according to NBHW from 1973, as

stillborn after 28 weeks of gestation. In 2008 NBWH changed their definition to stillborn after 22 weeks of gestation. Neonatal death was categorized into early neonatal death (within 6 days) and late neonatal death (7-27 days).

To investigate the relationship between HCV and the binary outcomes of pregnancy and births, adjusted odds ratio (aOR) were calculated through multivariate logistic regression. To estimate the association between HCV, gestational age and the Apgar score, adjusted odds ratios were calculated with linear regression.

For the models concerning gestational diabetes, hypertension, preeclampsia, caesarean section, still birth, early and late neonatal death, malformation and SGA, the following covariates were adjusted for: parity, alcohol use of the mother, drug use of the mother, diabetes and hypertension diagnoses prior to pregnancy, smoking, BMI of the mother, educational level of the mother and family situation. The covariate was included in the model if the interaction effect was more than 10%. P values <0.05 were considered to be statistically significant.

3.3.4 Paper IV

The study period was 1973-2011. Identification of all childbearing women with HCV infection and their infants was obtained by linking information on all the women reported to PHAS to the MBR, using their PIN. For this study we used the MBR and the DR. Information on outcomes regarding the infant was obtained from the MBR. Gestational age at birth was categorized into: very preterm (<32 weeks), moderately preterm (32-36 weeks), and term (≥37 weeks). The infants were then categorized if they were SGA. SGA was defined as birth-weight less than two standard deviations (SD) below the mean for gestational age based on Swedish reference curve of estimated fetal growth (290). Statistics Sweden added information on social economical support of the mother and her family at the time of birth.

As in paper III, the relevant ICD codes from ICD-10 (1997-2011), ICD-9 (1987-1996) and ICD-8 (1973-1986) were used for outcome of interest. ICD codes which constituted the main ICD-chapters were used to categorize cause of death. ICD codes of special interest (liver related, drug and alcohol related, accidents, Sudden Infant Death Syndrome (SIDS)) were then further analyzed. Information on date and cause of mortality of the children was obtained from the DR. The baseline characteristics between children of HCV-affected mothers (cases) and children of non-affected mothers (controls) were compared by reporting absolute and relative frequencies. Differences between the two groups were tested for with the chi-square test.

Survival time was analyzed. The end of follow-up, December 31, 2011, was considered an independent censoring event. Crude survival curves in the two groups were calculated with the Kaplan-Meier method. Crude and adjusted mortality HRs were estimated with proportional-hazard regression models. The crude model included the binary indicator for case (0 = control, 1 = case) as the only covariate. The adjusted models also included indicators for low gestational age, low birth weight, SGA, and smoking during pregnancy. P-values less than 0.05 were considered statistically significant.

3.4 ETHICAL APPROVALS

For all papers the personal identifiers were removed before the dataset was used for analysis. All studies were approved by The Regional Ethical Review Board in Stockholm according to the guidelines of the Helsinki Declaration.

4 RESULTS

4.1.1 Paper I

A total of 15,318 HBV infected patients were reported to SMI between the years 1990 and 2004. After exclusion of all acute HBV and all HCV co-infected individuals, the HBV cohort consisted of 9,464 subjects with chronic HBV-infection. The HBV study population contributed to overall 66,768 person-years of observation overall. 85% were born in 1950 or later and males accounted for 53%. A total of 76 patients were reported to the Cancer register with cancer originated in the liver. Of them, 31 patients were excluded because the cancer diagnosis was made before or within three months after the report of the HBV diagnosis. This left 45 patients for further risk analysis. The mean time between the HBV notification and the cancer diagnosis was 4.8 years. The SIRs are presented in table 1. The lifetime risk of developing HCC was estimated to be 10% (95% CI: 6-12)

Table 1. *SIRs for HCC (n=45) in HBV infected patients*

Years with HBV infection	Expected	Observed	SIR	95% CI
0-29	0.03	1	33	0.9-189
30-39	0.08	3	38	7-105
40-49	0.19	9	47	21-89
50-59	0.35	19	54	33-85
>60	0.65	13	20	11-34
Total	1.3	45	35	25-46

There were 3,238 patients reported with co-infection between 1990-2004. After excluding those with acute hepatitis B (n=1,850), the co-infection cohort consisted of 1,697 patients in the risk analysis. Observation time was 11,392 person-years. A total of 67% were born after 1950 and males accounted for 78%. In the co-infected cohort, 12 patients were diagnosed with HCC and reported to the Cancer register. Two of them were excluded because they were diagnosed with HCC within three months from diagnosis of HCV. This left 10 patients for further risk analysis. Median age of these patients was 58 years. The standardised incidence ratio for the stratum is expressed in table 2.

Table 2. *SIRs for HCC (n=10) in HBV and HCV co-infected patients*

Years with co-infection	Expected	Observed	SIR	95% CI
0-19	0.025	0	0	0-150
20-30	0.118	4	34	9-87
>30	0.066	6	91	33-198
Total	0.209	10	48	23-88

4.1.2 Paper II

The mean observation times per subject in the HBV cohort was 6.4 years, contributing to totally 60,697 person years. Mean age at death was 56 years, 68% were males. The most common route of transmission was neonatal transmission (80%). After excluding all individuals that died within six months from HBV notification there were 425 (4.5%) deaths. The all-cause mortality was significantly increased with SMR 2.3 in the HBV cohort. The most frequently underlying cause of death in the HBV cohort was neoplasm. The cause of death for all cohorts is presented in table 3.

The mean observation time of the HCV cohort was 6.3 years contributing to 214,602 person years. The mean age at death was 50 years and 77% were men. The most common route of transmission was IDU (57%) or unknown (32%). After excluding all deaths within six months from HCV notification (n=744) there were 4,651 deaths. The results are presented in table 3. The all-cause mortality was significantly increased with SMR 5.8 in the HCV cohort. The most frequent underlying cause of death in this cohort was external causes (e.g. injuries, intoxication, and suicide) which accounted for 29% of the deaths.

The mean time of observation in the HCV-HBV co-infection cohort was 7.9 years, in total 12,667 person years. The mean age at death was 44 years and 85% were male. The most common route of transmission was IDU (54%) or unknown (25%). There were 209 (13%) deaths in the co-infected cohort after excluding all deaths reported within six months from first HBV/HCV notification (n=21). The most frequently reported cause of death was the

same as for the HCV cohort or external causes (e.g. injuries, intoxication, and suicide), 34 %. The all-cause mortality was also significantly increased in this cohort with SMR 8.5.

In the HCV and the co-infected cohort, there was an excess risk for death from causes related to IDU, e.g. HIV, psychiatric diagnoses (98% drug related) and external reasons, compared with the general population. However, the relative risk of liver-related mortality was highly increased in all three cohorts.

Table 3. *Cause of death in the HBV, HCV and HBV-HCV cohorts. The risk is expressed as SMR, the observed deaths/expected deaths*

	HBV (311 deaths)		HCV (3,970 deaths)		HBV-HCV (188 deaths)	
Diagnosis	SMR*	95% CI	SMR*	95% CI	SMR*	95% cI
All Cause	2.3	2.0-2.6	5.8	5.6-6.0	8.5	7.3-9.8
Infection	13.7	8.7-20.6	28.7	25.2-32.5	44.8	25.1-74.0
Neoplasm	2.8	2.3-3.3	2.8	2.6-3.1	3.9	2.5-5.9
Blood/immune	6.3	0.8-22.6	30.6	22.6-40.6	19.6	0.5-109.2
Endocrine	1.8	0.7-3.8	5.6	4.5-6.9	6.6	1.8-17.1
Psychiatric	3.1	1.8-5.0	15.0	13.7-16.5	26.0	18.0-36.3
Circulatory	1.4	1.1-1.8	2.6	2.4-2.8	5.1	3.4-7.3
Digestive tract	5.8	3.8-8.5	15.3	13.8-16.9	16.5	9.0-27.6
External reasons	1.7	1.2-2.4	12.4	11.7-13.1	11.4	8.8-14.6
Subgroups						
Viral hepatitis	78.9	46.8-124.7	133.0	114.3-153.9	168.6	84.2-301.7
Liver cancer	31.2	21.9-43.2	34.9	30.1-40.2	65.2	33.7-113.9
Liver disease	10.7	6.8-15.9	25.1	22.3-28.0	24.4	13.0-41.8
All liver related	21.7	17.1-27.0	35.5	32.9-38.3	46.2	31.5-62,3
HIV	11.4	2.4-33.2	41.2	31.4-53.2	23.7	2.9-85.5
Alcohol,drug related	3.8	2.1-6.4	20.7	18.9-22.7	27.6	19.6-39-6

**SMR= observed/expected, the expected number of deaths were calculated using age and sex specific mortality rates in the general population*

All cause SMR related to age showed an excess mortality in all ages, but in the HCV and HBV-HCV cohorts the great excess mortality was at age 15-35, then slowly declining. The SMR by age showed that the excess mortality from liver related deaths increased with age. The maximum liver related SMR was 26 at age 60-69 in the HBV and 42 at age over 70 years in the HCV cohort.

The liver cancer mortality had the greatest excess risk with SMR 31.2 (HBV), 34.9 (HCV) and 65.2 (HBV-HCV). In the HCV cohort risk of death from cancer in lip/mouth, oesophagus, pancreas, larynx, lung, cervix uteri and kidney was also significantly increased.

4.1.3 Paper III

19,072 births to 9,599 mothers reported with HCV were included and 86,164 births of 83,986 mothers in whom HCV was never reported.

Complications of pregnancy are presented in table 4. There was no increased risk of preeclampsia, gestational diabetes or gestational hypertension in women with HCV infection. There was on the other hand a significantly increased risk of cholestatic liver disease during pregnancy. There was also a significantly increased risk of caesarean section in women notified with HCV infection compared with women in the general population.

Table 4. *Complications of pregnancy of mothers reported with HCV infection and of mothers of the comparison group, presented as Odd Ratio (OR)*

	HCV (n=19,072))	Non-HCV (n=86,164)	Crude OR	Adjusted OR
Gestational diabetes	**P=0.124**		1.18	0.88
No	18,970	85,739		
Yes	111	425		
Gestational hypertension	**P=0.218**		1.15	1.03
No	18,985	85,787		
Yes	96	377		
Preeclampsia	**P=0.846**		0.94	0.6
No	19,070	86,111		
Yes	11	53		
Cholestatis of pregnancy	**P=<0.001**		4.88	4.78
No	18,885	85,981		
Yes	196	183		
Fetal distress	**P= 0.250**		1.66	1.09
No	19,074	86,154		
Yes	7	19		

The pregnancy outcome is presented in table 5. The mean Apgar score after 5 minutes was significantly lower in infants to HCV-infected mothers compared to the comparison group. The mean difference in gestational age was also significantly lower in cases. The pregnancy of the mothers with HCV infection was at higher risk of terminating in stillbirth and there was also a significantly higher risk of late neonatal death than in the comparison group. Furthermore, we found an increased risk of neonatal seizure. There was neither increased risk of malformations nor of cephalohematoma, ventricle hemorrhage or fetal distress in the infants born to HCV infected mothers.

Table 5. *Pregnancy outcomes of mothers reported with HCV and of mothers of the comparison group, presented as Odds Ratio (OR)*

	HCV (n=19,072)	Non-HCV (n=86,164)	Crude OR	Adjusted OR
Cesarean section	**P< 0.001**		1.33	1.31
No	16,166	7,5907		
Yes	2915	10,257		
Small for gestational age	**P<0.001**		1.55	1.64
No	17,495	81,200		
Yes	885	2,657		
Missing	701	2307		
Stillbirth	**P<0.001**		6.62	7.03
No	18,934	86,063		
Yes	147	101		
Early neonatal death (1-6d)	**P=0.135**		1.31	1.41
No	19,041	86,026		
Yes	40	138		
Late neonatal death (7-27d)	**P=0.005**		1.91	1.78
No	19,054	86,100		
Yes	27	64		
Neonatal seizure	**P<0.005**		2.26	1.3
No	19,053	86,132		
Yes	28	32		

4.1.4 Paper IV

Overall, there were 438 (2.3%) deaths amongst the cases of 19,097 children to mothers with HCV infection, and 961 (1.1%) deaths in the comparison group.

Out of 438 deaths in total, 176 (39%) died in the first year of life, 110 (25%) at the age 1 to 20 years, 152 (34%) died between age 21 and 40 years. Out of the children, there were 688 that were reported with HCV to the PHAS. Of those were 127 individuals reported with HCV before the age of 2 years. In the comparison group, 241 individuals were reported with HCV during the study period. One of them was reported before the age of 2 years ($p<0.005$).

At infant up to one week of age, the mortality HR was 1.41 (95% CI 1.09-1.82) but when adjusted for low gestational age, low birth weight, SGA and smoking, the mortality HR was 0.98 (95% CI 0.73-1.31). From 1 to 4 weeks, the adjusted HR was 2.26 (95% CI 1.37-3.74, $p<0.05$) and from 1 to 6 months it was 2.63 (95% CI 1.88-3.69, $p<0.05$). Between the ages of 6 months to 15 years, there was no significant difference in mortality. Figure 4 shows the survival curves in the study population and the comparison group.

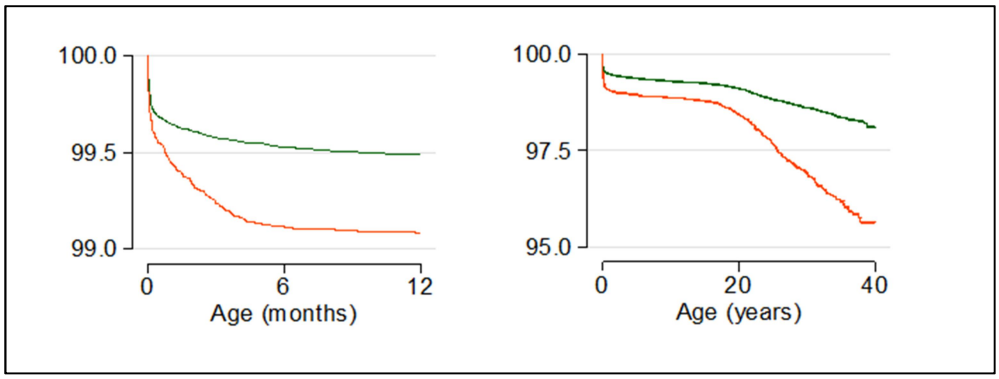

Figure 4. *Crude survival curves in the two groups with the Kaplan-Meier method*

Red line: Offspring of mothers with HCV infection

Green line: Offspring of mother without HCV infection

Children of HCV infected mothers died twice as often from sudden infant death syndrome (SIDS), older children died 1.6 times as often from accidents, and 2.7 times as often from drug associated deaths compared to the comparison group. There was no difference in cause of death by liver disease. Out of 129 of the children of HCV infected mothers that died of accidents, 65% were over the age of 21 years. All were over 1 year of age. After year 1995, death from SIDS was reduced in both cohorts. It was still more frequent in the HCV group compared to the comparison cohort.

5 DISCUSSION

5.1 METHODS AND STUDY DESIGN:

All the studies included in this thesis were register-based cohort studies. A cohort is a defined group of individuals followed for a period of time and can be defined by exposure, a specific experience or other specific characteristics. Such a cohort can then be compared to an unexposed population to investigate if there is an association between the exposure and the studied outcome. Cohort studies are well suited for rare outcomes, and can be performed using register data. In the current studies the exposure was mostly HCV but also hepatitis B and HCV and HBV co-infection. These cohorts were compared with the general population. The outcomes were HCCs, perinatal complications and mortality, which we studied using national registries.

To estimate the association between the virus infection and HCC in the first paper, the expected number of HCC was calculated on the basis of age, sex and calendar year-specific cancer incidence rates from the Cancer Register and the observed number of person years by age group and sex in the hepatitis cohorts. In the second paper, the association between hepatitis and various causes of death was analyzed by comparing the mortality of the hepatitis cohorts with the mortality of the general population by sex, age and calendar year-specific (year 1999) mortality rates of all diagnoses of interests. In paper 3, the association between HCV infection in mothers and various obstetrical and neonatal outcomes were investigated by comparing them with groups of five children matched by mother's age at birth, calendar year at birth, gender and county of birth and using aOR. Same method matching was used in paper 4 to assess of the association between HCV infection in mothers and the mortality of their offspring using HR.

Systematic errors

When studies are designed, attempt is always made to reduce potential systematic and random errors. Systematic errors are not affected by the size of the study population. Systematic errors are classified into three broad categories: selection bias, information bias and confounding.

Selection bias occurs when the association between exposure and disease differs for those individuals who participate and those who do not participate in the study.

In all papers in this thesis individuals reported with HCV and/or HBV infection during a specific period of time were included. In paper I an attempt was made to avoid selection bias of the HBV cohort by excluding individuals reported with acute HBV and all those reported with HBV infection in adulthood. Individuals presenting with HCC within three months after HBV notification were excluded from the analysis. This was done in order to minimize the effect of selection bias related to individuals being diagnosed with HBV at the time of the HCC diagnoses. In paper 2, the same approach was used; all individuals who died within six months after HCV and/or HBV notification were excluded. The exposed group may include people that were diagnosed and notified but then either had spontaneously cleared the infection or been treated and cured. This could certainly cause a selection bias and underestimation of the relative risk in all studies. In the unexposed group, there could be individuals with undiagnosed HCV or HBV infection or even individuals with HBV diagnosed before year 1990. but we believe these are only a few individuals that might somewhat dilute the results (291). It is estimated that approximately 99.1% of all diagnosed HBV and 99.5% of HCV infection are reported to the PHAS in Sweden,

Information bias occurs when information collected from or about the studied subject is incorrect. This can lead to the studied individual being placed in the incorrect category. Misclassification can be generalized or similar for both exposed and non-exposed groups and

thus are non-differential. If the information bias is related to exposure or outcome the misclassification becomes differential, thus differs in the exposed and unexposed group.

The diagnosis of chronic HBV and HCV infection is mainly based on random testing and screening of individuals at risk but both the physician and the laboratory are obliged to notify the infection to the PHAS since year 1990.

Misclassification of outcome may have occurred in our studies. In the Swedish Cancer register, it is estimated that 95% of all tumors diagnosed are reported and approximately 99% are verified histologically (278, 292). Two different radiological examinations for the diagnosis of HCC are required. Histological examination is however not required. In paper 1, 53 of 55 HCC were histologically verified. High sensitivity and specificity is established of specific diagnoses in the DR (279).

For registered diagnosis codes in the MBR and the PAR, misclassification of outcome could have occurred in both exposed and unexposed group. The same could apply to registered causes of death in the DR. In the current studies it could be argued that the potential misclassification might be similar in both exposed and un-exposed cohorts thus driving the difference in effect to null value.

Confounding

Confounding is a central issue for epidemiologic study design. Confounding is a factor that is closely associated with the exposure and a potential risk factor for the outcome. There are ways to modify a cohort study design to control the confounding variables.

Restriction is when a selection is made of subjects who have the same values for a confounder thus neutralizing the effect of the confounding in the outcome.

If a factor is suspected of being a possible confounder, a stratification of that factor could be used, for example, to stratify association between exposure and outcome per age group.

In paper 1, the risk of developing HCC in time/age intervals was stratified on estimated infection duration, thus controlling the effect of time on the development. In paper 4 the stratification of the risk regarding outcomes by route of infection was considered, but due to the large number of underreported and unknown route of infection, we chose not to do it.

Matching is a way to minimize the effect of confounding in cohort studies. Even though it is used to increase precision in studies, it should be used carefully as matching by more than one variable can introduce bias. In paper III and IV, 5 controls were selected from the background populations and matched by gender, year of birth, age of mother at birth and country of residence. It is probably preferable to have more than one control in the study to obtain a larger sample size to increase the precision of the results. Overall, 5 controls for every case were aimed for and resulted in 4.5 controls for every case.

If a confounder cannot be controlled in the study design, it is possible to control it through multivariate analysis which can potentially lead to more reliable results. There are confounders that are difficult to control because of information bias, when the patients e.g. do not disclose their use of illicit drugs when they visited the midwife. Well-known confounders are illicit drug use, alcohol consumption and smoking, which may have affected results in our studies. In paper III we attempted to adjust for these factors by linking to the patient register for all diagnoses revealing drug and alcohol abuse up to the date of birth.

In paper III, in which the outcomes gestational diabetes, hypertension, preeclampsia, caesarean section, still birth, early and late neonatal death, malformation and SGA were analysed, adjustment was made for the covariates: parity, alcohol use of the mother, drug abuse of the mother, diabetes and hypertension diagnoses prior to pregnancy, smoking, BMI of the mother, educational level of the mother and family situation. The covariate was included in the model if the interaction effect was more than 10%. We did not adjust for low

birth weight or short gestation, since according to our judgement it is in the causal pathway of the outcome.

In paper IV, models were used to adjust for low gestational age, low birth weight, SGA, and smoking during pregnancy primarily to explore the association between HCV infection of the mothers and SIDS. When considering the increased frequency of death by drugs abuse and alcohol overconsumption and accidents in teenage children of mothers with HCV infection, confounders might exist such as mother's drug abuse, poor social situation in relation to the mother's substance abuse, inherited behavior (drug abuse) and maybe inherited psychiatric diagnoses that we could not control for in the current studies (293). Further studies could explore the importance of these factors.

Random Error

Statistics are used to estimate the association between exposure and outcome after correction for confounding and to assess variability in the data. The goal of the analysis must be to obtain the most accurate results with minimal error.

Confidence interval (CI) consists of collected values around the estimated result indicating the quantity of random error in the estimate. A wide CI indicates a low precision in contrast to narrow CI indicating a high precision of the results. The size of the sample is one of the most important factors that determine the width of a CI. In our studies, we had a large sample size due to national registry cohorts and a long follow up time, generally generating narrow CIs, with high precision.

External and internal validity

Did our studies measure what was intended to be measured? Is the association between exposure and outcome properly demonstrated? In the absence of biases, confounding and random error it is stated that validity is high.

The ability to generalize our findings to other populations than the one studied is called external validity. In our studies we used participants with reported HCV and HBV infection and the children of women with HCV infection in Sweden. Our findings can only apply to HCV and HBV infected individuals. Confounding factors have been discussed above.

5.2 FINDINGS AND IMPLICATIONS

5.2.1 Hepatocellular carcinoma

This study demonstrated high risk for HCC in the HBV infected cohort after estimated 30 years of infection and in the HCV-HBV cohort after estimated 20 years of infection, compared to the general population in a low endemic area.

In the HBV cohort the relative risk for HCC gradually increased until it reached peak in the strata 50-59 years. A numerical decrease of SIR was then noticed after 60 years of infection. This may indicate that the most susceptible individuals have already developed their cancer by that time or perhaps the individuals in this stratum are under-diagnosed due to old age. High incidence in older ages is also expected in the general population and therefore could also explain the decrease in the relative risk in the stratum over 60 years of infection.

In the HBV-HCC co-infection cohort, the overall relative risk for developing HCC was 48 times elevated. Epidemiological studies on interaction between HBV and HCV infection have not been consistent. Earlier meta-analysis have demonstrated synergism or additive (supra-additive or even multiplicative) risk ratios of co-existence of the two viruses in HCC (140, 294). However a more recent meta-analysis presented a subadditive risk of the two viruses for HCC (141). Compared to a Swedish study on HCC among HCV infected individuals in Sweden the current study presented a numerically higher risk of developing HCC in dual infection compared to mono infection with either HBV or HCV indicating that there is an interference between the two viruses in the carcinogenic process (135). We could however not confirm this significantly because of too few HCC cases.

According to existing literature and to our findings, individuals with CHB and HBV-HCV dual infection are in excessive risk of developing HCC during their lifetime. The current study confirms that the HCV cohort and HBV-HCV cohort have the same characteristics and probably share the same hazardous lifestyles. These results might therefore support the decision and the Swedish Clinical guidelines and EASL to vaccinate individuals with confirmed chronic HCV against HBV to decrease the rate of cirrhosis and its complications (HCC) secondary to dual infection. (145).

5.2.2 Hepatitis C and/or B infection and mortality

The current national cohort study demonstrated that individuals with HCV and/or HBV infection had an excess risk of mortality from almost all diagnoses. The all-cause mortality in the HCV- and the co-infection cohorts was highest in the younger age group (15-35years). As noticed in paper I the HCV and HCV-HBV co infection cohorts consisted of a large proportion of people who inject drugs (PWIDS) either earlier or actively, often with concomitant alcohol use. This might be reflected in the high risk of death in younger ages from causes such as injuries, intoxication, suicide and external causes. This indicates that intravenous drug use and the associated lifestyle might be a larger threat than HCV infection in young drug addicts. Same trend was seen in the children of the women in the HCV cohort in paper IV, where there was an increased mortality after the age of 16 due to accidents and drug and alcohol abuse.

The HBV cohort consists mainly of individuals infected at birth or at early age in high endemic areas, as discussed earlier. The all-cause and liver related death in Sweden was very similar to these high endemic areas (254).

Mortality due to liver related causes was increased in all cohorts. The SMR due to liver related mortality gradually increased with age. The more prominent increase in the HCV and the co-infection cohort might be explained by concomitant alcohol induced disease. However

when the subgroup with HCV transmission via blood transfusion and the HBV cohort was analyzed where the increase in mortality due to psychiatric causes was less prominent, the SMR revealed increased death rates due to liver related causes as well.

The SMR due to HCC was increased in all cohorts 31, (HBV), 35 (HCV) and 65 (HCV-HBV). The excess SMR in the co-infection cohort could be explained by a synergy between the two viruses as discussed earlier.

The increased risk of death from congenital disease, genitourinary disease and renal failure reflects increased mortality due to underlying disease, which in turn by blood transfusion and hemodialysis results in the HCV transmission.

Increased mortality due to diabetes mellitus (DM) related causes in the HCV cohort were noticed. The association between DM and HCV has been described in earlier studies(295). This is in agreement with paper III, where the cohort of HCV infected mothers had significantly more often diabetes diagnoses before pregnancy compared with the general population.

Large proportion of the HCV and/or HBV cases were notified within 6 months of deaths that were due to lethal complications of the HCV and/or HBV infection. As discussed earlier the virus infection has a long lag time and can go unnoticed for decades until it presents in the terminal stages of the disease.

This study demonstrated and that all cohorts were at increased risk of mortality compared to the general population. The interaction of HCV-HBV Co-infection seems to entail increased mortality due to most causes of death. Attention should also be given to young individuals infected with HCV and their lifestyle for preventive interventions. In the light of the DAAs more effective screening in early stage of the disease is needed in risk groups with the aim of treatment before advanced liver disease develops.

5.2.3 Hepatitis C infection and pregnancy

Our study demonstrated that pregnancy in HCV infected women is associated with negative maternal and neonatal outcomes.

The present study demonstrated an increased risk for caesarean section in women with HCV infection compared to women in the general population. This might reflect different obstetrical management due to HCV infection but concern for adverse pregnancy outcomes and failed labor might be the most probable reasons. Concomitant HIV infection is also an indication for caesarean section, but after excluding HIV co-infected mothers from the analysis there was still an increased risk of caesarian section among HCV mono-infected women.

The study confirmed that women notified with HCV infection had increased rate of DM and diagnosis before birth. The association of HCV infection and DM has been described in several studies (154, 187, 296). Furthermore the study showed an increased risk for hypertension diagnosis before birth. One explanation might be that these women seek medical care more often because of co-morbidities and therefore get diagnosed earlier compared to the general population.

There was an increased risk of cholestatic liver disease during pregnancy (ICP). Risk factors for the development of ICP have been described. Several studies have shown a highly significant increase of ICP in women with HCV infection (173-175). Other have demonstrated that HCV infection is more prevalent in women with ICP (176). The etiology behind that is not clear. But several theories exist(171)

The present study demonstrated that infants born to HCV infected mothers were at significant risk of both preterm birth and being SGA. Earlier studies are in agreement with these results (297, 298). Proposed explanation might be through immunologic mechanisms. natural killer T (NKT) cells have been shown to be important for clearance of HCV in acute infection

(190). Hurtado and her group established that placental HCV infection increased cytotoxicity of NKT cells (191). This might explain the adverse birth outcomes in the HCV infected mothers as the infection of the virus induced NKT cytotoxicity which produces cytokines and stimulates the innate immune response in the mother, and thus contributes to placental damage, intrauterine growth restriction, perinatal mortality and preterm labor. A viral infection of the mother induced production of inflammatory cytokines and thus activated the maternal immune system that in turn caused placental damage, fetal mortality and preterm birth (192). If this process does not terminate the birth it might sensitize the mother to other infections and promote inflammation in the fetus (193).

The increased risk for preterm birth might also be explained by co-factors of the mother such as drug abuse and alcohol overconsumption. To minimize the effect of these co factors, adjustments were made for smoking, earlier drug use and alcohol use.

Increased risk of neonatal seizures was also demonstrated. This might in part be explained by neonatal abstinence syndrome in the children of mothers with active drug abuse. It is however of interest that there is a known biological basis for the association of HCV infection and neurological dysfunction in adults (157). The current findings are partially in accordance to results obtained by Salemi et al., who found borderline increase in neonatal seizures (299).

The current study demonstrated an increased risk for still-birth in women with HCV infection when compared to the general population. This might also have a multifactorial cause, being both attributed to the mother's abuse and influenced by the HCV inflammation. Studies evaluating intrauterine death have been contradictory, several have demonstrated no risk while other have demonstrated increased risk of spontaneous abortion mostly in acute hepatitis (297, 300). Advanced liver disease is a reported risk factor for perinatal death of infants (301, 302). Information about prior cirrhosis diagnosis of the mothers in the study was not obtained. This is however a rare occurrence. The risk of late neonatal death was also

significant. Reported data over neonatal mortality is lacking. The cause of deaths and potential mechanisms behind the increased risk of stillbirth and neonatal death needs to be explored in further studies.

The current study demonstrated that women with HCV are in increased risk of several adverse pregnancy outcomes. There was an excessive risk of perinatal mortality among infants to women with HCV infection. This might suggest that all women should be screened for HCV infection at the first neonatal visit. All women diagnosed and notified with HCV should be carefully monitored during pregnancy and birth. Women of fertile age might benefit from treatment for HCV infection with the aim of cure before pregnancy.

5.2.4 Survival of the offspring

Offspring of mothers with HCV infection had a significantly increased mortality rate compared to the children in the general population the first six month of life.

The study indicates that the most common cause of death was SIDS in infants less than 6 months of age. SIDS is one of the leading causes of death in infants (303, 304). Known risk factors for SIDS are smoking during pregnancy, preterm birth, metabolic abnormalities and malformation in the child (303). Earlier studies have also implicated drug use (methadone) during pregnancy as a risk factor (305). The incidence of SIDS has decreased in industrialized countries, including Sweden, since 1992 when the advice of change in sleeping positions of infants was issued. Our study shows that the number of SIDS decreased after 1995 in both cohorts, although it was still more frequent in children of HCV mothers. The mechanism of SIDS is not fully identified. Proposed mechanism might be HCV mediated injury during pregnancy although, parents that are noncompliant to the abovementioned advice is also plausible.

The mortality rate increased again after the age of 16 years. The study indicated the mortality was related to accidents and due to alcohol and drugs use. Same trend was seen in the HCV

cohort in paper II. Studies that have looked into cause of accidents in young adults have indicated that attention deficit hyperactivity disorder, insomnia and drug abuse and alcohol are possible risk factors (306-308). The impact of the maternal drug abuse probably being an important factor concerning alcohol and drug use in the children. A recent Swedish study demonstrated the influence of both genetic and parental factors contributing substantially to offspring tendency for drug abuse (293). This could in part be supported by the fact that 3% of the children of HCV infected mothers were reported to PHAS with HCV infection. This is significantly higher than in the general population. Information regarding co-morbidity of the children was not included in the study.

This study demonstrated that the offspring of women with HCV infection are at excessive risk of premature death, with significantly increased risks during the first six months after birth and in young adults. Mothers with HCV infection and their children should be carefully monitored with focus on the first six months of life to prevent premature deaths. Attention should also be given to their teenagers and their lifestyles, for preventive interventions.

6 IMPLICATIONS FOR FUTURE STUDIES

On the basis of the results of paper I- IV the following is of interest:

- To do a nationwide cohort study to analyse the association of HCV infection in mothers and morbidity in their children born after 1996.
- A prospective study of the outcome of pregnancy in HCV infected women. This should include clinical information about viremia, treatment (DAAs), co-morbidities, drug and alcohol abuse, smoking habits of the mother, and the HCV transmission rate with emphasis of perinatal morbidity and mortality.Data from regular monitoring of the fetus should be obtained in collaboration with a neonatolog and a obstetrician. This would further enhance our knowledge about pregnancy outcome in these women and the effect of treatment on the outcome.
- A prospective study of the children to mothers with HCV and HBV, including information about virus transmission, therapy of the mother, drug abuse, alcohol abuse, smoking habits of the mother and co-morbidities of the mother and the infant. This would further increase our understanding of the risk factors associated with the increased neonatal mortality in these children.

7 CONCLUSIONS

This thesis includes national register-based cohort studies of morbidity and mortality in children born to mothers with HCV infection and in patients with HCV and HBV infection in Sweden. The main conclusions are:

- There is a highly increased risk of HCC in individuals with chronic HBV infection and with HBV and HCV co-infection, when compared with the general population.
- All-cause mortality was significantly increased in the three cohorts of HBV, HCV and HCV-HBV co-infected. Liver-related mortality was highly increased in all three cohorts, especially after the age of 50 years. A large proportion of the HCV and/or HBV infections were notified within 6 months before death due to lethal complications. More effective screening to diagnose infection at an early stage is needed in risk groups with the aim to treat before complications such as cirrhos and HCC arise.
- In the HCV and HBV-HCV cohorts there was a high risk of lifestyle related mortality in relation to drug use, especially in young adults. Drug prevention intervention is needed for this groups.
- Pregnant women with HCV infection had an increased risk of adverse pregnancy and neonatal outcome. Screening for HCV should be considered to identify pregnant women with HCV. They should be carefully monitored through pregnancy and birth.
- An excessive perinatal mortality in infants of women with HCV infection was demonstrated. The cause of this is unclear and needs more research.
- Mortality was highly increased the first six month of life in the children of mothers with HCV infection. Our data indicated that SIDS was the main cause. Known risk factors for SIDS should be removed and the parents instructed to take measures to prevent SIDS.

- Mortality in children of mothers with HCV infection is significantly increased after age of 16, mostly due to drug and alcohol related causes and accidents. Mothers with HCV infection were often infected through IDU, which might increase the risk of drug use in their adolescent children. Awerness and sometimes intervention is needed for these children.

8 ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all those who made this thesis possible, in particular:

Professor Rolf Hultcrantz, my supervisor, for leading me through clinical work, research and life. Thank you for being so tirelessly supportive and patient with me. Your skillful and thoughtful guidance is an inspiration.

Professor Anders Ekbom, assistant supervisor, for sharing his brilliance, wisdom and wit. For always leaving his door open and finding time for discussion.

Associate Professor Ann-Sofi Duberg, assistant supervisor, for her guidance, support and all her valuable advices. For sharing her knowledge and experience in the field and for being an great inspiration.

Associate Professor Soo Aleman, assistant supervisor, for leading me through research and for sharing her knowledge, wisdom and friendship. Thank you for all the encouragement through the years.

Professor Matteo Bottai, professor and statistician, for fantastic collaboration and for teaching me statistics and for enduring all my numerous questions with great patience.

My co-authors Anna Törner, Erik Bäck, Karl Ekdahl, Ander Blaxhult, Åke Svensson for lending your expertise and contribution. Thank you for taking me on from beginning.

Francesca Bresso, my dear mentor, for being such a brilliant person and for being an inspiration, best friend, and for all the encouraging words through my years in the Karolinska Hospital

Jessica Filipsson, for being so fantastic and helpful with all possible problems.

Professor Einar Björnsson, for all his support, for giving me opportunity to finish my thesis and for his expert advice.

Anna Gudmundsdottir, for her expert advice and input.

Einar Leo Johannesson, Graphic designer, for all the help with my thesis.

Ragnar Befrits, my mentor and inspiration both in life and work.

.Michael Eberhardsson, for sharing with me all his knowledge in the field of Gastroenterology and for his valuable friendship.

Ann-Sofie Backman, for being a great inspiration and friend.

Maria Elmberg, for all the encouragement and for her friendship.

Jan Björk, for being such a brilliant, wise and empathic doctor and a friend. Always an inspiration.

Annika Olsson, Ingrid Ackzell, Annette Storåhls and Eva Berglund for being my friends in need in all my years in Stockholm and always lending an ear when I needed.

Peter Rosenberg for being a great inspiration and asking the important questions.

Leif Törkvist for all the encouragement.

All my co-workers in all of the departments of Gastrocentrum, I was truly blessed to have you as my work colleagues and friends. Miss you all!

Soley Omarsdottir and Halldora Skarphedinsdottir, for your wonderful and valuable support and friendship.

My parents for being my greatest inspirations in life and for all their assistance and encouragement.

My brothers for their endless support.

My children for their love and for challenging me no matter what.

Steinunn Þórðardóttir and Helga Eyjólfsdóttir for all the support and for keeping me sane during this period.

Halla Dóra Halldórsdóttir for all your support and encouraging words.

Álfheiður Ástvaldsdóttir and Sigga Björns, for your advice and friendship.

Signy Vala, Gudrun Bjork and Sunna for leading the way.

Björn Haraldsson, the love of my life, for always being there. I would never have achieved this without him.

All the rest of my family and friends, thank you for your support, help and patience, I am truly lucky to have you in my life.

9 REFERENCES

1. Papavramidou N, Fee E, Christopoulou-Aletra H. Jaundice in the Hippocratic Corpus. J Gastrointest Surg. 2007;11(12):1728-31.
2. Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV. Transfusion-associated hepatitis not due to viral hepatitis type A or B. N Engl J Med. 1975;292(15):767-70.
3. Mattsson L, Weiland O, Glaumann H. Long-term follow-up of chronic post-transfusion non-A, non-B hepatitis: clinical and histological outcome. Liver. 1988;8(3):184-8.
4. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science. 1989;244(4902):359-62.
5. Choo QL, Richman KH, Han JH, Berger K, Lee C, Dong C, et al. Genetic organization and diversity of the hepatitis C virus. Proc Natl Acad Sci U S A. 1991;88(6):2451-5.
6. Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science. 1989;244(4902):362-4.
7. Di Bisceglie AM, Goodman ZD, Ishak KG, Hoofnagle JH, Melpolder JJ, Alter HJ. Long-term clinical and histopathological follow-up of chronic posttransfusion hepatitis. Hepatology. 1991;14(6):969-74.
8. Mattsson L, Sonnerborg A, Weiland O. Outcome of acute symptomatic non-A, non-B hepatitis: a 13-year follow-up study of hepatitis C virus markers. Liver. 1993;13(5):274-8.
9. Catanese MT, Uryu K, Kopp M, Edwards TJ, Andrus L, Rice WJ, et al. Ultrastructural analysis of hepatitis C virus particles. Proc Natl Acad Sci U S A. 2013;110(23):9505-10.
10. Vieyres G, Dubuisson J, Pietschmann T. Incorporation of hepatitis C virus E1 and E2 glycoproteins: the keystones on a peculiar virion. Viruses. 2014;6(3):1149-87.
11. Major ME, Feinstone SM. The molecular virology of hepatitis C. Hepatology. 1997;25(6):1527-38.
12. Penin F, Dubuisson J, Rey FA, Moradpour D, Pawlotsky JM. Structural biology of hepatitis C virus. Hepatology. 2004;39(1):5-19.
13. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science. 1998;282(5386):103-7.
14. Zeuzem S. The kinetics of hepatitis C virus infection. Clin Liver Dis. 2001;5(4):917-30.
15. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59(1):318-27.

16. Kuiken C, Combet C, Bukh J, Shin IT, Deleage G, Mizokami M, et al. A comprehensive system for consistent numbering of HCV sequences, proteins and epitopes. Hepatology. 2006;44(5):1355-61.

17. Manns MP, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. Gut. 2006;55(9):1350-9.

18. Zein NN. Clinical significance of hepatitis C virus genotypes. Clin Microbiol Rev. 2000;13(2):223-35.

19. Maheshwari A, Thuluvath PJ. Management of acute hepatitis C. Clin Liver Dis. 2010;14(1):169-76; x.

20. Farci P, Alter HJ, Wong D, Miller RH, Shih JW, Jett B, et al. A long-term study of hepatitis C virus replication in non-A, non-B hepatitis. N Engl J Med. 1991;325(2):98-104.

21. Kamili S, Drobeniuc J, Araujo AC, Hayden TM. Laboratory diagnostics for hepatitis C virus infection. Clin Infect Dis. 2012;55 Suppl 1:S43-8.

22. Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. J Viral Hepat. 2001;8(2):87-95.

23. Pereira BJ, Levey AS. Hepatitis C virus infection in dialysis and renal transplantation. Kidney Int. 1997;51(4):981-99.

24. Lok AS, Chien D, Choo QL, Chan TM, Chiu EK, Cheng IK, et al. Antibody response to core, envelope and nonstructural hepatitis C virus antigens: comparison of immunocompetent and immunosuppressed patients. Hepatology. 1993;18(3):497-502.

25. Bouvier-Alias M, Patel K, Dahari H, Beaucourt S, Larderie P, Blatt L, et al. Clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. Hepatology. 2002;36(1):211-8.

26. Chevaliez S, Rodriguez C, Pawlotsky JM. New virologic tools for management of chronic hepatitis B and C. Gastroenterology. 2012;142(6):1303-13 e1.

27. Grant PR, Sims CM, Tedder RS. Quantification of HCV RNA levels and detection of core antigen in donations before seroconversion. Transfusion (Paris). 2002;42(8):1032-6.

28. Chevaliez S, Pawlotsky JM. How to use virological tools for optimal management of chronic hepatitis C. Liver Int. 2009;29 Suppl 1:9-14.

29. Sulkowski MS, Ray SC, Thomas DL. Needlestick transmission of hepatitis C. JAMA. 2002;287(18):2406-13.

30. Halfon P, Bourliere M, Penaranda G, Khiri H, Ouzan D. Real-time PCR assays for hepatitis C virus (HCV) RNA quantitation are adequate for clinical management of patients with chronic HCV infection. J Clin Microbiol. 2006;44(7):2507-11.

31. Pawlotsky JM, Bouvier-Alias M, Hezode C, Darthuy F, Remire J, Dhumeaux D. Standardization of hepatitis C virus RNA quantification. Hepatology. 2000;32(3):654-9.

32. Lavanchy D. The global burden of hepatitis C. Liver Int. 2009;29 Suppl 1:74-81.

33. Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and

circulation of hepatitis C virus genotypes. World J Gastroenterol. 2016;22(34):7824-40.

34. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4):1333-42.

35. Duberg A, Janzon R, Back E, Ekdahl K, Blaxhult A. The epidemiology of hepatitis C virus infection in Sweden. Euro Surveill. 2008;13(21).

36. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5(9):558-67.

37. Global burden of disease (GBD) for hepatitis C. J Clin Pharmacol. 2004;44(1):20-9.

38. Robaeys G, Rob B, Azar DG, Razavi H, Nevens F. Global genotype distribution of hepatitis C viral infection among people who inject drugs. J Hepatol. 2016.

39. Operskalski EA, Mosley JW, Tobler LH, Fiebig EW, Nowicki MJ, Mimms LT, et al. HCV viral load in anti-HCV-reactive donors and infectivity for their recipients. Transfusion (Paris). 2003;43(10):1433-41.

40. Vrielink H, Reesink HW, Zaaijer HL, Scholten E, Kremer LC, Cuypers HT, et al. Look-back of anti-HCV ELISA-positive, HCV-RNA PCR-negative donors and recipients of their blood products. Vox Sang. 1997;72(2):67-70.

41. Donahue JG, Munoz A, Ness PM, Brown DE, Jr., Yawn DH, McAllister HA, Jr., et al. The declining risk of post-transfusion hepatitis C virus infection. N Engl J Med. 1992;327(6):369-73.

42. Hutin YJ, Chen RT. Injection safety: a global challenge. Bull World Health Organ. 1999;77(10):787-8.

43. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, et al. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet. 2000;355(9207):887-91.

44. Widell A, Zhang YY, Andersson-Gare B, Hammarstrom L. At least three hepatitis C virus strains implicated in Swedish and Danish patients with intravenous immunoglobulin-associated hepatitis C. Transfusion (Paris). 1997;37(3):313-20.

45. Hauri AM, Armstrong GL, Hutin YJ. The global burden of disease attributable to contaminated injections given in health care settings. Int J STD AIDS. 2004;15(1):7-16.

46. Kucirka LM, Sarathy H, Govindan P, Wolf JH, Ellison TA, Hart LJ, et al. Risk of window period hepatitis-C infection in high infectious risk donors: systematic review and meta-analysis. Am J Transplant. 2011;11(6):1188-200.

47. Lidman C, Norden L, Kaberg M, Kall K, Franck J, Aleman S, et al. Hepatitis C infection among injection drug users in Stockholm Sweden: prevalence and gender. Scand J Infect Dis. 2009;41(9):679-84.

48. WHO. People Who Inject Drugs. World Health Organisation; 2016 [cited 2016 01-11-2016]. Available from: http://www.who.int/hiv/topics/idu/en/.

49. Hellard M, Sacks-Davis R, Gold J. Hepatitis C treatment for injection drug users: a review of the available evidence. Clin Infect Dis. 2009;49(4):561-73.

50. Wejstal R. Sexual transmission of hepatitis C virus. J Hepatol. 1999;31 Suppl 1:92-5.

51. Yeung LT, King SM, Roberts EA. Mother-to-infant transmission of hepatitis C virus. Hepatology. 2001;34(2):223-9.

52. Zaltron S, Spinetti A, Biasi L, Baiguera C, Castelli F. Chronic HCV infection: epidemiological and clinical relevance. BMC Infect Dis. 2012;12(2):1471-2334.

53. Gibb DM, Goodall RL, Dunn DT, Healy M, Neave P, Cafferkey M, et al. Mother-to-child transmission of hepatitis C virus: evidence for preventable peripartum transmission. Lancet. 2000;356(9233):904-7.

54. Mok J, Pembrey L, Tovo PA, Newell ML. When does mother to child transmission of hepatitis C virus occur? Arch Dis Child Fetal Neonatal Ed. 2005;90(2):F156-60.

55. Polywka S, Pembrey L, Tovo PA, Newell ML. Accuracy of HCV-RNA PCR tests for diagnosis or exclusion of vertically acquired HCV infection. J Med Virol. 2006;78(2):305-10.

56. Benova L, Mohamoud YA, Calvert C, Abu-Raddad LJ. Vertical transmission of hepatitis C virus: systematic review and meta-analysis. Clin Infect Dis. 2014;59(6):765-73.

57. Cottrell EB, Chou R, Wasson N, Rahman B, Guise JM. Reducing risk for mother-to-infant transmission of hepatitis C virus: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 2013;158(2):109-13.

58. A significant sex--but not elective cesarean section--effect on mother-to-child transmission of hepatitis C virus infection. J Infect Dis. 2005;192(11):1872-9.

59. Mast EE, Hwang LY, Seto DS, Nolte FS, Nainan OV, Wurtzel H, et al. Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. J Infect Dis. 2005;192(11):1880-9.

60. Gudrun Lindh GA, Björn Fischler, Anders Eilard, Anders Widell. Kunskapcentrum för infektioner under graviditet. 2016.

61. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol. 2011;55(2):245-64.

62. Spera AM, Eldin TK, Tosone G, Orlando R. Antiviral therapy for hepatitis C: Has anything changed for pregnant/lactating women? World J Hepatol. 2016;8(12):557-65.

63. Duberg AS, Pettersson H, Aleman S, Blaxhult A, Daviethsdottir L, Hultcrantz R, et al. The burden of hepatitis C in Sweden: a national study of inpatient care. J Viral Hepat. 2011;18(2):106-18.

64. Duberg AS, Hansdotter F, How AL, Holmstrom A, Lesko B. [Important with generous sampling for hepatitis C after blood transfusion. The National Board of Health and Welfare's new recommendation for risk groups]. Lakartidningen. 2013;110(34-35):1477-9. Angelaget med generosare provtagning for hepatit C efter blodtransfusion. Socialstyrelsens nya rekommendation for riskgrupper.

65. The Public Health Service of Sweden P. Skattning av antalet personer som lever med en hepatit C-infektion i Sverige 2015. In: Folkhälsomyndigheten., editor. 2016.

66. Bruggmann P, Berg T, Ovrehus AL, Moreno C, Brandao Mello CE, Roudot-Thoraval F, et al. Historical epidemiology of hepatitis C virus (HCV) in selected countries. J Viral Hepat. 2014;21 Suppl 1:5-33.

67. Duberg AS, Blach S, Falconer K, Kaberg M, Razavi H, Aleman S. The future disease burden of hepatitis C virus infection in Sweden and the impact of different treatment strategies. Scand J Gastroenterol. 2015;50(2):233-44.

68. Lindh M, Hannoun C. Genotyping of hepatitis C virus by Taqman real-time PCR. J Clin Virol. 2005;34(2):108-14.

69. Busch K, Waldenstrom J, Lagging M, Aleman S, Weiland O, Kovamees J, et al. Prevalence and comorbidities of chronic hepatitis C: a nationwide population-based register study in Sweden. Scand J Gastroenterol. 2016:1-8.

70. Folkhälsomyndigheten. Public Health Agency of Sweden H, Hepatitis C statistics 2014. Hepatitis C statistics 2016. 2016. Available from: https://www.folkhalsomyndigheten.se/.

71. The nordic liver transplant registry. 2016 [updated 15.8.2016]. Available from: http://www.scandiatransplant.org/.

72. Shimizu YK, Weiner AJ, Rosenblatt J, Wong DC, Shapiro M, Popkin T, et al. Early events in hepatitis C virus infection of chimpanzees. Proc Natl Acad Sci U S A. 1990;87(16):6441-4.

73. Abe K, Inchauspe G, Shikata T, Prince AM. Three different patterns of hepatitis C virus infection in chimpanzees. Hepatology. 1992;15(4):690-5.

74. Bassett SE, Guerra B, Brasky K, Miskovsky E, Houghton M, Klimpel GR, et al. Protective immune response to hepatitis C virus in chimpanzees rechallenged following clearance of primary infection. Hepatology. 2001;33(6):1479-87.

75. Hoofnagle JH. Course and outcome of hepatitis C. Hepatology. 2002;36(5 Suppl 1):S21-9.

76. Cox AL, Netski DM, Mosbruger T, Sherman SG, Strathdee S, Ompad D, et al. Prospective evaluation of community-acquired acute-phase hepatitis C virus infection. Clin Infect Dis. 2005;40(7):951-8.

77. Aach RD, Stevens CE, Hollinger FB, Mosley JW, Peterson DA, Taylor PE, et al. Hepatitis C virus infection in post-transfusion hepatitis. An analysis with first- and second-generation assays. N Engl J Med. 1991;325(19):1325-9.

78. Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo QL, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med. 1989;321(22):1494-500.

79. Farci P, Alter HJ, Shimoda A, Govindarajan S, Cheung LC, Melpolder JC, et al. Hepatitis C virus-associated fulminant hepatic failure. N Engl J Med. 1996;335(9):631-4.

80. Chu CM, Yeh CT, Liaw YF. Fulminant hepatic failure in acute hepatitis C: increased risk in chronic carriers of hepatitis B virus. Gut. 1999;45(4):613-7.

81. Hoofnagle JH, Carithers RL, Jr., Shapiro C, Ascher N. Fulminant hepatic failure: summary of a workshop. Hepatology. 1995;21(1):240-52.

82. Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, et al. The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. N Engl J Med. 1992;327(27):1899-905.

83. Barrera JM, Bruguera M, Ercilla MG, Gil C, Celis R, Gil MP, et al. Persistent hepatitis C viremia after acute self-limiting posttransfusion hepatitis C. Hepatology. 1995;21(3):639-44.

84. Barreiro P, Labarga P, Fernandez-Montero JV, Poveda E, de Mendoza C, Sanchez C, et al. Longitudinal changes in viral RNA concentration in patients with chronic hepatitis C and/or HIV infection in the absence of antiviral therapy. J Clin Virol. 2013;58(2):391-5.

85. Villano SA, Vlahov D, Nelson KE, Cohn S, Thomas DL. Persistence of viremia and the importance of long-term follow-up after acute hepatitis C infection. Hepatology. 1999;29(3):908-14.

86. Lehmann M, Meyer MF, Monazahian M, Tillmann HL, Manns MP, Wedemeyer H. High rate of spontaneous clearance of acute hepatitis C virus genotype 3 infection. J Med Virol. 2004;73(3):387-91.

87. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, et al. The natural history of hepatitis C virus infection: host, viral, and environmental factors. JAMA. 2000;284(4):450-6.

88. Mir HM, Stepanova M, Afendy M, Kugelmas M, Younossi ZM. African americans are less likely to have clearance of hepatitis C virus infection: the findings from recent U.S. population data. J Clin Gastroenterol. 2012;46(8):e62-5.

89. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009;461(7265):798-801.

90. Tillmann HL, Thompson AJ, Patel K, Wiese M, Tenckhoff H, Nischalke HD, et al. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. Gastroenterology. 2010;139(5):1586-92, 92 e1.

91. Thursz M, Yallop R, Goldin R, Trepo C, Thomas HC. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. Lancet. 1999;354(9196):2119-24.

92. Kaplan M, Gawrieh S, Cotler SJ, Jensen DM. Neutralizing antibodies in hepatitis C virus infection: a review of immunological and clinical characteristics. Gastroenterology. 2003;125(2):597-604.

93. Haberstroh A, Schnober EK, Zeisel MB, Carolla P, Barth H, Blum HE, et al. Neutralizing host responses in hepatitis C virus infection target viral entry at postbinding steps and membrane fusion. Gastroenterology. 2008;135(5):1719-28 e1.

94. Grakoui A, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghrayeb J, et al. HCV persistence and immune evasion in the absence of memory T cell help. Science. 2003;302(5645):659-62.

95. Vogt M, Klostermann B, Braun S, Busch R, Hess J, Frosner G, et al. Prevalence and clinical role of GBV-C infection after cardiac surgery in childhood: a study on 414 patients. J Infect. 2006;53(1):43-8.

96. Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology. 2003;125(1):80-8.

97. Merican I, Sherlock S, McIntyre N, Dusheiko GM. Clinical, biochemical and histological features in 102 patients with chronic hepatitis C virus infection. Q J Med. 1993;86(2):119-25.

98. Rodger AJ, Jolley D, Thompson SC, Lanigan A, Crofts N. The impact of diagnosis of hepatitis C virus on quality of life. Hepatology. 1999;30(5):1299-301.

99. Ware JE, Jr., Bayliss MS, Mannocchia M, Davis GL. Health-related quality of life in chronic hepatitis C: impact of disease and treatment response. The Interventional Therapy Group. Hepatology. 1999;30(2):550-5.

100. Shakil AO, Conry-Cantilena C, Alter HJ, Hayashi P, Kleiner DE, Tedeschi V, et al. Volunteer blood donors with antibody to hepatitis C virus: clinical, biochemical, virologic, and histologic features. The Hepatitis C Study Group. Ann Intern Med. 1995;123(5):330-7.

101. Sagnelli E, Pisaturo M, Stanzione M, Messina V, Alessio L, Sagnelli C, et al. Clinical presentation, outcome, and response to therapy among patients with acute exacerbation of chronic hepatitis C. Clin Gastroenterol Hepatol. 2013;11(9):1174-80 e11.

102. Yano M, Kumada H, Kage M, Ikeda K, Shimamatsu K, Inoue O, et al. The long-term pathological evolution of chronic hepatitis C. Hepatology. 1996;23(6):1334-40.

103. Tong MJ, el-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med. 1995;332(22):1463-6.

104. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology. 1998;28(6):1687-95.

105. Rodger AJ, Roberts S, Lanigan A, Bowden S, Brown T, Crofts N. Assessment of long-term outcomes of community-acquired hepatitis C infection in a cohort with sera stored from 1971 to 1975. Hepatology. 2000;32(3):582-7.

106. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, et al. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology. 1990;12(4 Pt 1):671-5.

107. Zarski JP, Mc Hutchison J, Bronowicki JP, Sturm N, Garcia-Kennedy R, Hodaj E, et al. Rate of natural disease progression in patients with chronic hepatitis C. J Hepatol. 2003;38(3):307-14.

108. Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med. 1999;340(16):1228-33.

109. Wiese M, Grungreiff K, Guthoff W, Lafrenz M, Oesen U, Porst H. Outcome in a hepatitis C (genotype 1b) single source outbreak in Germany--a 25-year multicenter study. J Hepatol. 2005;43(4):590-8.

110. Wiese M, Fischer J, Lobermann M, Gobel U, Grungreiff K, Guthoff W, et al. Evaluation of liver disease progression in the German hepatitis C virus (1b)-contaminated anti-D cohort at 35 years after infection. Hepatology. 2014;59(1):49-57.

111. Levine RA, Sanderson SO, Ploutz-Snyder R, Murray F, Kay E, Hegarty J, et al. Assessment of fibrosis progression in untreated irish women with chronic hepatitis C

contracted from immunoglobulin anti-D. Clin Gastroenterol Hepatol. 2006;4(10):1271-7.

112. Xu F, Moorman AC, Tong X, Gordon SC, Rupp LB, Lu M, et al. All-Cause Mortality and Progression Risks to Hepatic Decompensation and Hepatocellular Carcinoma in Patients Infected With Hepatitis C Virus. Clin Infect Dis. 2016;62(3):289-97.

113. Dienstag JL, Ghany MG, Morgan TR, Di Bisceglie AM, Bonkovsky HL, Kim HY, et al. A prospective study of the rate of progression in compensated, histologically advanced chronic hepatitis C. Hepatology. 2011;54(2):396-405.

114. Garcia-Alvarez M, Pineda-Tenor D, Jimenez-Sousa MA, Fernandez-Rodriguez A, Guzman-Fulgencio M, Resino S. Relationship of vitamin D status with advanced liver fibrosis and response to hepatitis C virus therapy: a meta-analysis. Hepatology. 2014;60(5):1541-50.

115. Freedman ND, Everhart JE, Lindsay KL, Ghany MG, Curto TM, Shiffman ML, et al. Coffee intake is associated with lower rates of liver disease progression in chronic hepatitis C. Hepatology. 2009;50(5):1360-9.

116. Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. Hepatology. 2004;39(4):1147-71.

117. Singh S, Fujii LL, Murad MH, Wang Z, Asrani SK, Ehman RL, et al. Liver stiffness is associated with risk of decompensation, liver cancer, and death in patients with chronic liver diseases: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2013;11(12):1573-84 e1-2; quiz e88-9.

118. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. Gastroenterology. 2012;142(6):1293-302 e4.

119. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology. 1997;112(2):463-72.

120. Belli LS, Berenguer M, Cortesi PA, Strazzabosco M, Rockenschaub SR, Martini S, et al. Delisting of liver transplant candidates with chronic hepatitis C after viral eradication: A European study. J Hepatol. 2016;65(3):524-31.

121. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011;365(12):1118-27.

122. Bosetti C, Levi F, Boffetta P, Lucchini F, Negri E, La Vecchia C. Trends in mortality from hepatocellular carcinoma in Europe, 1980-2004. Hepatology. 2008;48(1):137-45.

123. Ryerson AB, Eheman CR, Altekruse SF, Ward JW, Jemal A, Sherman RL, et al. Annual Report to the Nation on the Status of Cancer, 1975-2012, featuring the increasing incidence of liver cancer. Cancer. 2016;122(9):1312-37.

124. Hashim D, Boffetta P, La Vecchia C, Rota M, Bertuccio P, Malvezzi M, et al. The global decrease in cancer mortality: trends and disparities. Ann Oncol. 2016;27(5):926-33.

125. El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? Hepatology. 2014;60(5):1767-75.

126. Kalaitzakis E, Gunnarsdottir SA, Josefsson A, Bjornsson E. Increased risk for malignant neoplasms among patients with cirrhosis. Clin Gastroenterol Hepatol. 2011;9(2):168-74.

127. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006;45(4):529-38.

128. Schutte K, Bornschein J, Malfertheiner P. Hepatocellular carcinoma--epidemiological trends and risk factors. Dig Dis. 2009;27(2):80-92.

129. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127(5 Suppl 1):S35-50.

130. Yang JD, Kim WR, Coelho R, Mettler TA, Benson JT, Sanderson SO, et al. Cirrhosis is present in most patients with hepatitis B and hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2011;9(1):64-70.

131. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295(1):65-73.

132. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med. 2002;347(3):168-74.

133. Chen JD, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, et al. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. Gastroenterology. 2010;138(5):1747-54.

134. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol. 2008;49(4):652-7.

135. Strauss R, Torner A, Duberg AS, Hultcrantz R, Ekdahl K. Hepatocellular carcinoma and other primary liver cancers in hepatitis C patients in Sweden - a low endemic country. J Viral Hepat. 2008;15(7):531-7.

136. Hu KQ, Tong MJ. The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. Hepatology. 1999;29(4):1311-6.

137. Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. Hepatology. 1997;26(3 Suppl 1):34S-8S.

138. El-Serag HB. Hepatocellular carcinoma: recent trends in the United States. Gastroenterology. 2004;127(5 Suppl 1):S27-34.

139. Torner A, Stokkeland K, Svensson A, Dickman PW, Hultcrantz R, Montgomery S, et al. The underreporting of hepatocellular carcinoma to the Cancer Register and a log-linear model to estimate a more correct incidence. Hepatology. 2016.

140. Shi J, Zhu L, Liu S, Xie WF. A meta-analysis of case-control studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma in China. Br J Cancer. 2005;92(3):607-12.

141. Cho LY, Yang JJ, Ko KP, Park B, Shin A, Lim MK, et al. Coinfection of hepatitis B and C viruses and risk of hepatocellular carcinoma: systematic review and meta-analysis. Int J Cancer. 2011;128(1):176-84.

142. Yu MW, You SL, Chang AS, Lu SN, Liaw YF, Chen CJ. Association between hepatitis C virus antibodies and hepatocellular carcinoma in Taiwan. Cancer Res. 1991;51(20):5621-5.

143. Benvegnu L, Fattovich G, Noventa F, Tremolada F, Chemello L, Cecchetto A, et al. Concurrent hepatitis B and C virus infection and risk of hepatocellular carcinoma in cirrhosis. A prospective study. Cancer. 1994;74(9):2442-8.

144. Amin J, O'Connell D, Bartlett M, Tracey E, Kaldor J, Law M, et al. Liver cancer and hepatitis B and C in New South Wales, 1990-2002: a linkage study. Aust N Z J Public Health. 2007;31(5):475-82.

145. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908-43.

146. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology. 1998;28(3):751-5.

147. Cacoub P, Renou C, Rosenthal E, Cohen P, Loury I, Loustaud-Ratti V, et al. Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC. Groupe d'Etude et de Recherche en Medecine Interne et Maladies Infectieuses sur le Virus de l'Hepatite C. Medicine (Baltimore). 2000;79(1):47-56.

148. Lunel F, Musset L, Cacoub P, Frangeul L, Cresta P, Perrin M, et al. Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage. Gastroenterology. 1994;106(5):1291-300.

149. Gisbert JP, Garcia-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in B-cell non-Hodgkin's lymphoma: systematic review and meta-analysis. Gastroenterology. 2003;125(6):1723-32.

150. Chiao EY, Engels EA, Kramer JR, Pietz K, Henderson L, Giordano TP, et al. Risk of immune thrombocytopenic purpura and autoimmune hemolytic anemia among 120 908 US veterans with hepatitis C virus infection. Arch Intern Med. 2009;169(4):357-63.

151. Gisbert JP, Garcia-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in porphyria cutanea tarda: systematic review and meta-analysis. J Hepatol. 2003;39(4):620-7.

152. Bianchi FB. Autoimmune hepatitis: the lesson of the discovery of hepatitis C virus. J Hepatol. 1993;18(3):273-5.

153. Johnson RJ, Willson R, Yamabe H, Couser W, Alpers CE, Wener MH, et al. Renal manifestations of hepatitis C virus infection. Kidney Int. 1994;46(5):1255-63.

154. White DL, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. J Hepatol. 2008;49(5):831-44.

155. Antonelli A, Ferri C, Pampana A, Fallahi P, Nesti C, Pasquini M, et al. Thyroid disorders in chronic hepatitis C. Am J Med. 2004;117(1):10-3.

156. Petta S, Maida M, Macaluso FS, Barbara M, Licata A, Craxi A, et al. Hepatitis C Virus Infection Is Associated With Increased Cardiovascular Mortality: A Meta-Analysis of Observational Studies. Gastroenterology. 2016;150(1):145-55 e4; quiz e15-6.

157. Monaco S, Ferrari S, Gajofatto A, Zanusso G, Mariotto S. HCV-related nervous system disorders. Clin Dev Immunol. 2012;236148(10):30.

158. Amin J, Law MG, Bartlett M, Kaldor JM, Dore GJ. Causes of death after diagnosis of hepatitis B or hepatitis C infection: a large community-based linkage study. Lancet. 2006;368(9539):938-45.

159. Pinchoff J, Drobnik A, Bornschlegel K, Braunstein S, Chan C, Varma JK, et al. Deaths among people with hepatitis C in New York City, 2000-2011. Clin Infect Dis. 2014;58(8):1047-54.

160. Mahajan R, Xing J, Liu SJ, Ly KN, Moorman AC, Rupp L, et al. Mortality among persons in care with hepatitis C virus infection: the Chronic Hepatitis Cohort Study (CHeCS), 2006-2010. Clin Infect Dis. 2014;58(8):1055-61.

161. Ly KN, Xing J, Klevens RM, Jiles RB, Ward JW, Holmberg SD. The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. Ann Intern Med. 2012;156(4):271-8.

162. Neal KR, Ramsay S, Thomson BJ, Irving WL. Excess mortality rates in a cohort of patients infected with the hepatitis C virus: a prospective study. Gut. 2007;56(8):1098-104.

163. Omland LH, Krarup H, Jepsen P, Georgsen J, Harritshoj LH, Riisom K, et al. Mortality in patients with chronic and cleared hepatitis C viral infection: a nationwide cohort study. J Hepatol. 2010;53(1):36-42.

164. Walter SR, Thein HH, Amin J, Gidding HF, Ward K, Law MG, et al. Trends in mortality after diagnosis of hepatitis B or C infection: 1992-2006. J Hepatol. 2011;54(5):879-86.

165. Innes H, McDonald S, Hayes P, Dillon JF, Allen S, Goldberg D, et al. Mortality in hepatitis C patients who achieve a sustained viral response compared to the general population. J Hepatol. 2016.

166. Seeff LB, Hollinger FB, Alter HJ, Wright EC, Cain CM, Buskell ZJ, et al. Long-term mortality and morbidity of transfusion-associated non-A, non-B, and type C hepatitis: A National Heart, Lung, and Blood Institute collaborative study. Hepatology. 2001;33(2):455-63.

167. Le Campion A, Larouche A, Fauteux-Daniel S, Soudeyns H. Pathogenesis of hepatitis C during pregnancy and childhood. Viruses. 2012;4(12):3531-50.

168. Alter MJ. Hepatitis C virus infection in the United States. J Hepatol. 1999;31 Suppl 1:88-91.

169. Arshad M, El-Kamary SS, Jhaveri R. Hepatitis C virus infection during pregnancy and the newborn period--are they opportunities for treatment? J Viral Hepat. 2011;18(4):229-36.

170. Zahran KM, Badary MS, Agban MN, Abdel Aziz NH. Pattern of hepatitis virus infection among pregnant women and their newborns at the Women's Health Center of Assiut University, Upper Egypt. Int JGynaecol Obstet. 2010;111(2):171-4.

171. Floreani A, Paternoster D, Zappala F, Cusinato R, Bombi G, Grella P, et al. Hepatitis C virus infection in pregnancy. Br J Obstet Gynaecol. 1996;103(4):325-9.

172. Hsieh TY, Yu CH, Kuo PL, Chang FM. Acute viral hepatitis C-induced jaundice in pregnancy. Taiwan J Obstet Gynecol. 2006;45(2):180-2.

173. Locatelli A, Roncaglia N, Arreghini A, Bellini P, Vergani P, Ghidini A. Hepatitis C virus infection is associated with a higher incidence of cholestasis of pregnancy. Br J Obstet Gynaecol. 1999;106(5):498-500.

174. Paternoster DM, Fabris F, Palu G, Santarossa C, Bracciante R, Snijders D, et al. Intrahepatic cholestasis of pregnancy in hepatitis C virus infection. Acta Obstet Gynecol Scand. 2002;81(2):99-103.

175. Ropponen A, Sund R, Riikonen S, Ylikorkala O, Aittomaki K. Intrahepatic cholestasis of pregnancy as an indicator of liver and biliary diseases: a population-based study. Hepatology. 2006;43(4):723-8.

176. Marschall HU, Wikstrom Shemer E, Ludvigsson JF, Stephansson O. Intrahepatic cholestasis of pregnancy and associated hepatobiliary disease: a population-based cohort study. Hepatology. 2013;58(4):1385-91.

177. Paternoster DM, Santarossa C, Grella P, Palu G, Baldo V, Boccagni P, et al. Viral load in HCV RNA-positive pregnant women. Am J Gastroenterol. 2001;96(9):2751-4.

178. Ohto H, Terazawa S, Sasaki N, Hino K, Ishiwata C, Kako M, et al. Transmission of hepatitis C virus from mothers to infants. The Vertical Transmission of Hepatitis C Virus Collaborative Study Group. N Engl J Med. 1994;330(11):744-50.

179. Conte D, Fraquelli M, Prati D, Colucci A, Minola E. Prevalence and clinical course of chronic hepatitis C virus (HCV) infection and rate of HCV vertical transmission in a cohort of 15,250 pregnant women. Hepatology. 2000;31(3):751-5.

180. Gervais A, Bacq Y, Bernuau J, Martinot M, Auperin A, Boyer N, et al. Decrease in serum ALT and increase in serum HCV RNA during pregnancy in women with chronic hepatitis C. J Hepatol. 2000;32(2):293-9.

181. Jabeen T, Cannon B, Hogan J, Crowley M, Devereux C, Fanning L, et al. Pregnancy and pregnancy outcome in hepatitis C type 1b. QJM. 2000;93(9):597-601.

182. Fontaine H, Nalpas B, Carnot F, Brechot C, Pol S. Effect of pregnancy on chronic hepatitis C: a case-control study. Lancet. 2000;356(9238):1328-9.

183. Vento S, Longhi MS, Cainelli F, Concia E. Effect of pregnancy on chronic hepatitis C. Lancet. 2001;357(9253):389-90.

184. Oketani M, Shibatou T, Yamashita K, Arima T. Postpartum acute exacerbation of chronic hepatitis C with complete response to interferon-alpha. J Gastroenterol. 2002;37(8):658-62.

185. Latt NC, Spencer JD, Beeby PJ, McCaughan GW, Saunders JB, Collins E, et al. Hepatitis C in injecting drug-using women during and after pregnancy. J Gastroenterol Hepatol. 2000;15(2):175-81.

186. Hillemanns P, Dannecker C, Kimmig R, Hasbargen U. Obstetric risks and vertical transmission of hepatitis C virus infection in pregnancy. Acta Obstet Gynecol Scand. 2000;79(7):543-7.

187. Pergam SA, Wang CC, Gardella CM, Sandison TG, Phipps WT, Hawes SE. Pregnancy complications associated with hepatitis C: data from a 2003-2005 Washington state birth cohort. Am J Obstet Gynecol. 2008;199(1):38 e1-9.

188. Huang QT, Hang LL, Zhong M, Gao YF, Luo ML, Yu YH. Maternal HCV infection is associated with intrauterine fetal growth disturbance: A meta-analysis of observational studies. Medicine (Baltimore). 2016;95(35):e4777.

189. Huang QT, Huang Q, Zhong M, Wei SS, Luo W, Li F, et al. Chronic hepatitis C virus infection is associated with increased risk of preterm birth: a meta-analysis of observational studies. J Viral Hepat. 2015;22(12):1033-42.

190. Golden-Mason L, Castelblanco N, O'Farrelly C, Rosen HR. Phenotypic and functional changes of cytotoxic CD56pos natural T cells determine outcome of acute hepatitis C virus infection. J Virol. 2007;81(17):9292-8.

191. Hurtado CW, Golden-Mason L, Brocato M, Krull M, Narkewicz MR, Rosen HR. Innate immune function in placenta and cord blood of hepatitis C--seropositive mother-infant dyads. PLoS One. 2010;5(8):e12232.

192. Koga K, Cardenas I, Aldo P, Abrahams VM, Peng B, Fill S, et al. Activation of TLR3 in the trophoblast is associated with preterm delivery. Am J Reprod Immunol. 2009;61(3):196-212.

193. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. Am J Reprod Immunol. 2010;63(6):425-33.

194. Woods R. Long-term trends in fetal mortality: implications for developing countries. Bull World Health Organ. 2008;86(6):460-6.

195. Flenady V, Middleton P, Smith GC, Duke W, Erwich JJ, Khong TY, et al. Stillbirths: the way forward in high-income countries. Lancet. 2011;377(9778):1703-17.

196. Gissler M, Mohangoo AD, Blondel B, Chalmers J, Macfarlane A, Gaizauskiene A, et al. Perinatal health monitoring in Europe: results from the EURO-PERISTAT project. Inform Health Soc Care. 2010;35(2):64-79.

197. Froen JF, Gordijn SJ, Abdel-Aleem H, Bergsjo P, Betran A, Duke CW, et al. Making stillbirths count, making numbers talk - issues in data collection for stillbirths. BMC Pregnancy Childbirth. 2009;9:58.

198. Man J, Hutchinson JC, Heazell AE, Ashworth M, Levine S, Sebire NJ. Stillbirth and intrauterine fetal death: factors affecting determination of cause of death at autopsy. Ultrasound Obstet Gynecol. 2016;25(10):16016.

199. Luo ZC, Senecal S, Simonet F, Guimond E, Penney C, Wilkins R. Birth outcomes in the Inuit-inhabited areas of Canada. CMAJ. 2010;182(3):235-42.

200. Bateman BT, Simpson LL. Higher rate of stillbirth at the extremes of reproductive age: a large nationwide sample of deliveries in the United States. Am J Obstet Gynecol. 2006;194(3):840-5.

201. Getahun D, Ananth CV, Selvam N, Demissie K. Adverse perinatal outcomes among interracial couples in the United States. Obstet Gynecol. 2005;106(1):81-8.

202. Salihu HM, Kinniburgh BA, Aliyu MH, Kirby RS, Alexander GR. Racial disparity in stillbirth among singleton, twin, and triplet gestations in the United States. Obstet Gynecol. 2004;104(4):734-40.

203. Ravelli AC, Tromp M, Eskes M, Droog JC, van der Post JA, Jager KJ, et al. Ethnic differences in stillbirth and early neonatal mortality in The Netherlands. J Epidemiol Community Health. 2011;65(8):696-701.

204. Jorgensen T, Mortensen LH, Andersen AM. Social inequality in fetal and perinatal mortality in the Nordic countries. Scand J Public Health. 2008;36(6):635-49.

205. Flenady V, Koopmans L, Middleton P, Froen JF, Smith GC, Gibbons K, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. Lancet. 2011;377(9774):1331-40.

206. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ. Selected major risk factors and global and regional burden of disease. Lancet. 2002;360(9343):1347-60.

207. Aliyu MH, Lynch O, Nana PN, Alio AP, Wilson RE, Marty PJ, et al. Alcohol consumption during pregnancy and risk of placental abruption and placenta previa. Matern Child Health J. 2011;15(5):670-6.

208. Yudkin PL, Wood L, Redman CW. Risk of unexplained stillbirth at different gestational ages. Lancet. 1987;1(8543):1192-4.

209. Smith GC. Life-table analysis of the risk of perinatal death at term and post term in singleton pregnancies. Am J Obstet Gynecol. 2001;184(3):489-96.

210. Haavaldsen C, Sarfraz AA, Samuelsen SO, Eskild A. The impact of maternal age on fetal death: does length of gestation matter? Am J Obstet Gynecol. 2010;203(6):554 e1-8.

211. Man J, Hutchinson JC, Heazell AE, Ashworth M, Levine S, Sebire NJ. Stillbirth and intrauterine fetal death: factors affecting determination of cause of death at autopsy. Ultrasound Obstet Gynecol. 2016.

212. Money D, Boucoiran I, Wagner E, Dobson S, Kennedy A, Lohn Z, et al. Obstetrical and neonatal outcomes among women infected with hepatitis C and their infants. J Obstet Gynaecol Can. 2014;36(9):785-94.

213. Resti M, Jara P, Hierro L, Azzari C, Giacchino R, Zuin G, et al. Clinical features and progression of perinatally acquired hepatitis C virus infection. J Med Virol. 2003;70(3):373-7.

214. Tovo PA, Pembrey LJ, Newell ML. Persistence rate and progression of vertically acquired hepatitis C infection. European Paediatric Hepatitis C Virus Infection. J Infect Dis. 2000;181(2):419-24.

215. Yeung LT, To T, King SM, Roberts EA. Spontaneous clearance of childhood hepatitis C virus infection. J Viral Hepat. 2007;14(11):797-805.

216. Bortolotti F, Resti M, Giacchino R, Azzari C, Gussetti N, Crivellaro C, et al. Hepatitis C virus infection and related liver disease in children of mothers with antibodies to the virus. J Pediatr. 1997;130(6):990-3.

217. Palomba E, Manzini P, Fiammengo P, Maderni P, Saracco G, Tovo PA. Natural history of perinatal hepatitis C virus infection. Clin Infect Dis. 1996;23(1):47-50.

218. Fischler B. Hepatitis C virus infection. Semin Fetal Neonatal Med. 2007;12(3):168-73.

219. Lakemedelsverket. Läkemedelsbehandling av Hepatit C-virusinfektion hos vuxna och barn- behandlingsrekommendation. . 15th of April 2015 ed.

220. Swain MG, Lai MY, Shiffman ML, Cooksley WG, Zeuzem S, Dieterich DT, et al. A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferon alfa-2a and ribavirin. Gastroenterology. 2010;139(5):1593-601.

221. Reichard O, Glaumann H, Fryden A, Norkrans G, Wejstal R, Weiland O. Long-term follow-up of chronic hepatitis C patients with sustained virological response to alpha-interferon. J Hepatol. 1999;30(5):783-7.

222. Veldt BJ, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, et al. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. Ann Intern Med. 2007;147(10):677-84.

223. Aleman S, Rahbin N, Weiland O, Davidsdottir L, Hedenstierna M, Rose N, et al. A risk for hepatocellular carcinoma persists long-term after sustained virologic response

in patients with hepatitis C-associated liver cirrhosis. Clin Infect Dis. 2013;57(2):230-6.

224. Hedenstierna M, Weiland O, Brass A, Bankwitz D, Behrendt P, Uhnoo I, et al. Long-term follow-up of successful hepatitis C virus therapy: waning immune responses and disappearance of liver disease are consistent with cure. Aliment Pharmacol Ther. 2015;41(6):532-43.

225. Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, Peters M, et al. Treatment of chronic non-A,non-B hepatitis with recombinant human alpha interferon. A preliminary report. N Engl J Med. 1986;315(25):1575-8.

226. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med. 1998;339(21):1485-92.

227. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet. 1998;352(9138):1426-32.

228. Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med. 2000;343(23):1666-72.

229. Lindsay KL, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, et al. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology. 2001;34(2):395-403.

230. Bourliere M, Ouzan D, Rosenheim M, Doffoel M, Marcellin P, Pawlotsky JM, et al. Pegylated interferon-alpha2a plus ribavirin for chronic hepatitis C in a real-life setting: the Hepatys French cohort (2003-2007). Antivir Ther. 2012;17(1):101-10.

231. Syed E, Rahbin N, Weiland O, Carlsson T, Oksanen A, Birk M, et al. Pegylated interferon and ribavirin combination therapy for chronic hepatitis C virus infection in patients with Child-Pugh Class A liver cirrhosis. Scand J Gastroenterol. 2008;43(11):1378-86.

232. Bourliere M, Khaloun A, Wartelle-Bladou C, Oules V, Portal I, Benali S, et al. Future treatment of patients with HCV cirrhosis. Liver Int. 2012;32 Suppl 1:113-9.

233. Sarrazin C, Kieffer TL, Bartels D, Hanzelka B, Muh U, Welker M, et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology. 2007;132(5):1767-77.

234. Poordad F, Dieterich D. Treating hepatitis C: current standard of care and emerging direct-acting antiviral agents. J Viral Hepat. 2012;19(7):449-64.

235. Janusinfo. Nationellt införande av nya läkemedel Hepatit C. Janusinfo; 2015.

236. EASL Recommendations on Treatment of Hepatitis C 2016. J Hepatol. 2016.

237. Lawitz E, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, et al. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. Lancet. 2014;384(9956):1756-65.

238. Jacobson IM, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, Sulkowski MS, et al. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. N Engl J Med. 2013;368(20):1867-77.

239. Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med. 2013;368(20):1878-87.

240. Zeuzem S, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, et al. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. N Engl J Med. 2014;370(21):1993-2001.

241. Osinusi A, Meissner EG, Lee YJ, Bon D, Heytens L, Nelson A, et al. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients with unfavorable treatment characteristics: a randomized clinical trial. JAMA. 2013;310(8):804-11.

242. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med. 2014;370(3):211-21.

243. Afdhal N, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, et al. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med. 2014;370(20):1889-98.

244. Afdhal N, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, et al. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. N Engl J Med. 2014;370(16):1483-93.

245. Manns M, Pol S, Jacobson IM, Marcellin P, Gordon SC, Peng CY, et al. All-oral daclatasvir plus asunaprevir for hepatitis C virus genotype 1b: a multinational, phase 3, multicohort study. Lancet. 2014;384(9954):1597-605.

246. Everson GT, Sims KD, Rodriguez-Torres M, Hezode C, Lawitz E, Bourliere M, et al. Efficacy of an interferon- and ribavirin-free regimen of daclatasvir, asunaprevir, and BMS-791325 in treatment-naive patients with HCV genotype 1 infection. Gastroenterology. 2014;146(2):420-9.

247. Poordad F, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, et al. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. N Engl J Med. 2014;370(21):1973-82.

248. Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med. 2004;350(11):1118-29.

249. Wieland SF, Spangenberg HC, Thimme R, Purcell RH, Chisari FV. Expansion and contraction of the hepatitis B virus transcriptional template in infected chimpanzees. Proc Natl Acad Sci U S A. 2004;101(7):2129-34.

250. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology. 2004;126(7):1750-8.

251. Levrero M, Pollicino T, Petersen J, Belloni L, Raimondo G, Dandri M. Control of cccDNA function in hepatitis B virus infection. J Hepatol. 2009;51(3):581-92.

252. Pollicino T, Squadrito G, Cerenzia G, Cacciola I, Raffa G, Craxi A, et al. Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection. Gastroenterology. 2004;126(1):102-10.

253. Neuveut C, Wei Y, Buendia MA. Mechanisms of HBV-related hepatocarcinogenesis. J Hepatol. 2010;52(4):594-604.

254. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004;11(2):97-107.

255. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet. 2015;386(10003):1546-55.

256. Mast EE, Weinbaum CM, Fiore AE, Alter MJ, Bell BP, Finelli L, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: immunization of adults. MMWR Recomm Rep. 2006;55(RR-16):1-33; quiz CE1-4.

257. Edmunds WJ, Medley GF, Nokes DJ, O'Callaghan CJ, Whittle HC, Hall AJ. Epidemiological patterns of hepatitis B virus (HBV) in highly endemic areas. Epidemiol Infect. 1996;117(2):313-25.

258. Kiire CF. The epidemiology and prophylaxis of hepatitis B in sub-Saharan Africa: a view from tropical and subtropical Africa. Gut. 1996;38 Suppl 2:S5-12.

259. Hoffmann G, Berglund G, Elmstahl S, Eriksson S, Verbaan H, Widell A, et al. Prevalence and clinical spectrum of chronic viral hepatitis in a middle-aged Swedish general urban population. Scand J Gastroenterol. 2000;35(8):861-5.

260. Stenkvist J, Lidbrink P, Olofsson I, von Sydow M, Lindh G. Hepatitis B seroprevalence in persons attending youth clinics in Stockholm, Sweden in 2008. Int J STD AIDS. 2012;23(11):767-71.

261. Struve J, Giesecke J, Olcen P, von Sydow M, Weiland O. Prevalence of hepatitis B virus markers in Sweden: a community-based serosurvey of 4,000 young Swedish adults. Am J Epidemiol. 1992;135(4):409-17.

262. Christenson B, Bottiger M, Grillner L. The prevalence of hepatitis B in Sweden; a statistical serosurvey of 3381 Swedish inhabitants. Epidemiol Infect. 1997;119(2):221-5.

263. Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med. 1981;94(6):744-8.

264. Realdi G, Alberti A, Rugge M, Bortolotti F, Rigoli AM, Tremolada F, et al. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology. 1980;79(2):195-9.

265. Lok AS. Natural history and control of perinatally acquired hepatitis B virus infection. Dig Dis. 1992;10(1):46-52.

266. Chang MH, Hwang LY, Hsu HC, Lee CY, Beasley RP. Prospective study of asymptomatic HBsAg carrier children infected in the perinatal period: clinical and liver histologic studies. Hepatology. 1988;8(2):374-7.

267. Lok AS, Lai CL. A longitudinal follow-up of asymptomatic hepatitis B surface antigen-positive Chinese children. Hepatology. 1988;8(5):1130-3.

268. Lok AS, Lai CL, Wu PC, Leung EK, Lam TS. Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. Gastroenterology. 1987;92(6):1839-43.

269. Liaw YF, Chu CM, Su IJ, Huang MJ, Lin DY, Chang-Chien CS. Clinical and histological events preceding hepatitis B e antigen seroconversion in chronic type B hepatitis. Gastroenterology. 1983;84(2):216-9.

270. Wong GL, Chan HL, Yu Z, Chan HY, Tse CH, Wong VW. Liver fibrosis progression is uncommon in patients with inactive chronic hepatitis B: a prospective cohort study with paired transient elastography examination. J Gastroenterol Hepatol. 2013;28(12):1842-8.

271. Zoulim F, Lebosse F, Levrero M. Current treatments for chronic hepatitis B virus infections. Curr Opin Virol. 2016;18:109-16.

272. Tyson GL, Kramer JR, Duan Z, Davila JA, Richardson PA, El-Serag HB. Prevalence and predictors of hepatitis B virus coinfection in a United States cohort of hepatitis C virus-infected patients. Hepatology. 2013;58(2):538-45.

273. Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. N Engl J Med. 1999;341(1):22-6.

274. Raimondo G, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. J Hepatol. 2007;46(1):160-70.

275. Torbenson M, Thomas DL. Occult hepatitis B. Lancet Infect Dis. 2002;2(8):479-86.

276. Caccamo G, Saffioti F, Raimondo G. Hepatitis B virus and hepatitis C virus dual infection. World J Gastroenterol. 2014;20(40):14559-67.

277. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncol. 2009;48(1):27-33.

278. Mattsson B, Wallgren A. Completeness of the Swedish Cancer Register. Non-notified cancer cases recorded on death certificates in 1978. Acta Radiol Oncol. 1984;23(5):305-13.

279. Socialstyrelsen NBoHaW. Dödsorsakstatistik. 2010 ed2010. p. 38.

280. Socialstyrelsen TNBoHaW. Bortfall och kvalitet. 2016 [cited 2016 2016-11-01]. Available from: http://www.socialstyrelsen.se/register/dodsorsaksregistret/bortfallochkvalitet.

281. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC Public Health. 2011;11:450.

282. Cnattingius S, Ericson A, Gunnarskog J, Kallen B. A quality study of a medical birth registry. Scand J Soc Med. 1990;18(2):143-8.

283. Socialstyrelsen NBoHaW. The Swedish Medical Birth register.

284. Duberg AS, Nordstrom M, Torner A, Reichard O, Strauss R, Janzon R, et al. Non-Hodgkin's lymphoma and other nonhepatic malignancies in Swedish patients with hepatitis C virus infection. Hepatology. 2005;41(3):652-9.

285. Davidsdottir L, Duberg AS, Torner A, Aleman S, Back E, Ekdahl K, et al. Hepatocellular carcinoma in individuals with HBV infection or HBV-HCV co-infection in a low endemic country. Scand J Gastroenterol. 2010;45(7-8):944-52.

286. Weiland O, Berg JV, Bjorvatn B, Flehmig B, Lundbergh P. Acute viral hepatitis A, B and non-A, non-B in Stockholm in the 1950s and 1970s: a comparison. Infection. 1981;9(6):268-74.

287. Blackberg J, Braconier JH, Widell A, Kidd-Ljunggren K. Long-term outcome of acute hepatitis B and C in an outbreak of hepatitis in 1969-72. Eur J Clin Microbiol Infect Dis. 2000;19(1):21-6.

288. Mansson AS, Moestrup T, Nordenfelt E, Widell A. Continued transmission of hepatitis B and C viruses, but no transmission of human immunodeficiency virus among intravenous drug users participating in a syringe/needle exchange program. Scand J Infect Dis. 2000;32(3):253-8.

289. Stokkeland K, Ebrahim F, Hultcrantz R, Ekbom A, Stephansson O. Mothers with alcoholic liver disease and the risk for preterm and small-for-gestational-age birth. Alcohol Alcohol. 2013;48(2):166-71.

290. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta paediatrica (Oslo, Norway : 1992). 1996;85(7):843-8.

291. Jansson A, Arneborn M, Ekdahl K. Sensitivity of the Swedish statutory surveillance system for communicable diseases 1998-2002, assessed by the capture-recapture method. Epidemiol Infect. 2005;133(3):401-7.

292. Mattsson B. Cancer Registration in Sweden:studies on completeness and validity of incidence and mortality register. Karolinska institutet, Stockholm, Sweden2008.

293. Kendler KS, Ohlsson H, Sundquist K, Sundquist J. The causes of parent-offspring transmission of drug abuse: a Swedish population-based study. Psychol Med. 2015;45(1):87-95.

294. Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer. 1998;75(3):347-54.

295. Mihm S. Hepatitis C virus, diabetes and steatosis: clinical evidence in favor of a linkage and role of genotypes. Dig Dis. 2010;28(1):280-4.

296. Caronia S, Taylor K, Pagliaro L, Carr C, Palazzo U, Petrik J, et al. Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. Hepatology. 1999;30(4):1059-63.

297. Pergam SA, Wang CC, Gardella CM, Sandison TG, Phipps WT, Hawes SE. Pregnancy complications associated with hepatitis C: data from a 2003-2005 Washington state birth cohort. Am J Obstet Gynecol. 2008;199(1):19.

298. Connell LE, Salihu HM, Salemi JL, August EM, Weldeselasse H, Mbah AK. Maternal hepatitis B and hepatitis C carrier status and perinatal outcomes. Liver Int. 2011;31(8):1163-70.

299. Salemi JL, Whiteman VE, August EM, Chandler K, Mbah AK, Salihu HM. Maternal hepatitis B and hepatitis C infection and neonatal neurological outcomes. J Viral Hepat. 2014;21(11):e144-53.

300. Medhat A, el-Sharkawy MM, Shaaban MM, Makhlouf MM, Ghaneima SE. Acute viral hepatitis in pregnancy. Int JGynaecol Obstet. 1993;40(1):25-31.

301. Rasheed SM, Abdel Monem AM, Abd Ellah AH, Abdel Fattah MS. Prognosis and determinants of pregnancy outcome among patients with post-hepatitis liver cirrhosis. Int JGynaecol Obstet. 2013;121(3):247-51.

302. Shaheen AA, Myers RP. The outcomes of pregnancy in patients with cirrhosis: a population-based study. Liver Int. 2010;30(2):275-83.

303. Fleming PJ, Blair PS, Pease A. Sudden unexpected death in infancy: aetiology, pathophysiology, epidemiology and prevention in 2015. Arch Dis Child. 2015;100(10):984-8.

304. Monti MC, Borrelli P, Nosetti L, Taje S, Perotti M, Bonarrigo D, et al. Incidence of apparent life-threatening events and post-neonatal risk factors. Acta Paediatr. 2016.

305. Cohen MC, Morley SR, Coombs RC. Maternal use of methadone and risk of sudden neonatal death. Acta Paediatr. 2015;104(9):883-7.

306. Philip P, Micoulaud-Franchi JA, Lagarde E, Taillard J, Canel A, Sagaspe P, et al. Attention Deficit Hyperactivity Disorder Symptoms, Sleepiness and Accidental Risk in 36140 Regularly Registered Highway Drivers. PLoS One. 2015;10(9):e0138004.

307. London AS, Landes SD. Attention Deficit Hyperactivity Disorder and adult mortality. Prev Med. 2016;90:8-10.

308. Mokdad AH, Forouzanfar MH, Daoud F, Mokdad AA, El Bcheraoui C, Moradi-Lakeh M, et al. Global burden of diseases, injuries, and risk factors for young people's health during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2016;387(10036):2383-401.